MYOCARDIAL PYRUVATE METABOLISM DURING HEMORRHAGIC SHOCK

Jerry Nagler

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MYOCARDIAL PYRUVATE METABOLISM DURING HEMORRHAGIC SHOCK

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DEDICATED TO:

My Parents

and

Much Hard Work

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INTRODUCTION

The pathogenesis of hemorrhagic shock is acute severe blood loss. The consequences of this blood loss affect every organ system to some extent. The interaction of organ systems is so complex, and our present understanding of patho-physiology in this area is so meager that despite the voluminous literature published on the subject, there is still no unifying theory to explain all of the pathologic mechanisms occurring during hemorrhagic shock.

Remington et al.[1] have studied the most obvious aspect of hemorrhagic shock, the circulatory response. Thirty dogs were bled in stages to a mean arterial pressure of fifty millimeters mercury. The observations on the animals' circulatory systems were divided into three phases. In phase one, the arterial systolic and diastolic blood pressures fell, the pulse pressure narrowed, the heart rate did not increase (thus cardiac output fell), and the peripheral resistance increased slightly. In phase two, the diastolic pressure remained constant although the mean blood pressure fell as the pulse pressure decreased, the heart rate increased (so did cardiac output), and peripheral resistance again increased slightly. In phase three, arterial blood pressure fell rapidly, peripheral resistance decreased, cardiac output decreased, the heart size increased terminally

while the central venous pressure remained constant or increased, and all dogs died. Remington's group concluded that by phase three the arterioles became so hypoxic as to become unresponsive to vasoconstrictive influences, leading to a fatal expansion of the arterial tree.

The concept of ultimate peripheral vascular failure in shock has been accepted and extended by some groups to explain the most puzzling aspect of hemorrhagic shock, irreversible shock. It is well known that beyond a certain time, a re-infusion of lost blood will not prevent continued cardiovascular deterioration. If dogs are kept in a state of hemorrhagic shock long enough, it has been observed that they will spontaneously auto-transfuse themselves by sucking in shed blood which has been held in reservoirs. This phenomenon is presumed to be due to a failure to maintain a fixed arterial blood pressure and is taken by most experimenters to be the earliest indication of irreversible shock. Weidner et al.[2] bled twenty-seven dogs to a mean arterial blood pressure of twenty-five to thirty millimeters mercury and kept the pressure at this level until the dogs auto-transfused some of their shed blood. At this point, the authors re-infused all of the remaining blood. Using a strain gauge to measure the force of myocardial contraction, they found that except for the period just prior to death, the blood pressures fell after re-infusion of all lost blood while the myocardial force remained normal or nearly normal.

Rothe and Selkurt,[3] using the same criteria for irreversible shock, found that despite repeated transfusions the central venous pressures of their shocked dogs fell. They[4] also claim to have discovered vasodilator substances in portal and arterial blood during the terminal hypotensive phase of hemorrhagic shock. Although they concede the presence of terminal cardiac failure, they ascribe the cause of irreversible shock to peripheral failure with decreased cardiac filling.

Frank et al.[5] took another approach and found that after bleeding dogs to a mean arterial blood pressure of thirty millimeters mercury they could prevent the death of the dogs by perfusing the hepatic circulatory system at a normal pressure. Perfusing just the jugular veins did not increase survival and so they concluded that liver damage is the cause of irreversible shock. Exactly how liver damage results in irreversible shock is not clear from their work, but they do claim that hepectomized dogs resemble dogs in hemorrhagic shock. They note that in early phases of shock serum non-protein nitrogen (mostly amino acids) is elevated, blood fibrinogen and prothrombin are reduced and plasma protein regeneration is impaired. All of these observations are indications of decreased liver function. Furthermore, the rate of survival was found to be inversely proportional to the rate of amino acid rise in the serum.

Lillehei et al.,[8] by concentrating on the intestines, have developed yet another theory to explain the patho-physiology of hemorrhagic shock. They claim that selective perfusion of neither the liver nor any other body section except the mesenteric circulatory system will prevent irreversible shock. Dogs were bled to a mean arterial pressure of thirty-five millimeters mercury and maintained at this pressure until spontaneous auto-transfusion began. Then all remaining shed blood was returned by the authors. During the initial hypotensive period the flow to the gut was found to be reduced by ninety per cent, more than any other organ. The period of auto-transfusion was marked by a change from intense mesenteric vasoconstriction to vasodilitation and pooling of blood in the mesenteric capillary beds. In the post-transfusion (irreversible) phase, there was increased pooling of blood in the mesentery with congestion of small bowel mucosa and bloody diarrhea. The authors claim that occlusion of the superior mesenteric artery in normal dogs for three hours will duplicate the picture of reversible shock while occlusion for four to five hours will re-create irreversible shock. They propose that the prolonged life of post-capillary sphincters as compared to pre-capillary sphincters enables the fatal pooling of blood in the gut to occur. The role of gut bacteria and endotoxins is ruled out by the observation that pre-sterilization of the gastro-

intestinal tract does not help. Irreversible shock is believed to be related to the inherent properties of the mesenteric vessels.

Schweinberg et al.[9] also concentrated on the role of intestinal damage during hemorrhagic shock and also noted swollen hemorrhagic mucosa and bloody diarrhea, but ascribed these lesions to damage created by bacterial endotoxins and not shock per se. Dogs pre-treated with non-absorbable antibiotics, polymixin B or bacitracin, were able to survive longer in shock and did not spontaneously auto-transfuse even after six hours. Blood or plasma taken from dogs and rabbits during irreversible shock produced no harmful effects in normal dogs and rabbits but when given to animals during the stage of reversible shock (early hemorrhagic shock), bloody diarrhea and early death occurred. Since the infused plasma was found to be sterile, the authors concluded that an endotoxin derived from endogenous gut bacteria produces irreversible shock because of the inability of animals to detoxify the endotoxin during early hemorrhagic shock. Jacob et al.[10] found that pre-treatment of dogs with either aureomycin, neomycin, terramycin or penicillin (orally or parenterally) increased per cent survival from twenty to fifty per cent. Clostridia and various intestinal aerobes were found in twenty-five per cent of shock tissues and blood cultures. Since they found that virtual elimination of all intra-intestinal flora alone did not affect

survival but systemic antibiotic coverage did, these authors attributed success with antibiotic therapy to the elimination of bacteria within tissues during shock.

Another logical organ to study in relation to irreversible shock is the heart. For, if the pump fails, then changes in the rest of the circulatory system become extraneous in determining the final outcome. Many researchers have taken the approach that cardiac failure preceeds or at least contributes to circulatory failure during hemorrhagic shock.

Crowell and Guyton[11,12] bled fifty-five dogs to a mean arterial blood pressure of thirty millimeters mercury and kept the dogs at this pressure until the stage of irreversibility (which they defined as the period in which the dogs began to auto-transfuse). They found: a) no change in oxygen consumption, b) no change in peripheral resistance if hematocrit and blood pressure were kept constant, c) suddenly increased left atrial pressure and then increased right atrial pressure. The requirement of increased atrial (or venous) pressure to maintain cardiac output is characteristic of heart failure. The authors propose that in early hemorrhagic shock no blood infusion is needed to maintain the low arterial pressures characteristic of this stage, and the heart is not in failure. At the stage of auto-transfusion, increased blood volume is needed to maintain arterial blood pressure as the heart is in early failure.

Finally, in later stages, the failure becomes severe and no amount of blood infusion can enable the heart to maintain an adequate arterial pressure. Wiggers[13] too found that in late hemorrhagic shock cardiac output could only be maintained at the expense of elevated central venous pressures. Sarnoff et al.[14] discovered that the increase in left atrial pressure could be prevented by increased flow through the left main coronary artery and concluded that insufficient left coronary flow is responsible for the deterioration occurring during late hemorrhagic shock. Albert et al.[15] bled dogs to a mean arterial blood pressure of thirty millimeters mercury and found increased survival following a period of hypotension and low cardiac output (500 cc/min.) if the coronary arteries were selectively perfused. Glaviano and Klovda[16] ascribe decreased cardiac performance in late hemorrhagic shock to depletion of norepinephrine stores in the sympathetic nerve endings in the heart secondary to constant sympathetic stimulation. A gradual decrease in systolic and diastolic blood pressures, left ventricular pressure and force of contraction in response to stellate ganglion stimulation was noted. Lefer et al.[17] have characterized a "mycardial depressant factor" in hemorrhagic shock. Plasma taken from cats in hemorrhagic shock showed a decreasing inotropic effect on isolated papillary muscle contractility. There was virtually a linear relationship between the concentration of shock

dialysate and the magnitude of diminished muscle contractility. Further studies revealed the factor to be a small dialyzable peptide of molecular weight eight hundred to one thousand (believed to be too small to be an endotoxin). The concentration of this factor in serum is proportional to the length of time the animal is hypotensive. Lefer[18] was able to demonstrate that in dogs bled to a mean arterial blood pressure of forty millimeters mercury until twenty per cent of shed blood was auto-transfused, dialysis resulted in increased cardiac output, decreased myocardial depressant factor in the blood stream and increased survival. The author's hypothesis is that hypotensive ischemia results in decreased splanchnic circulation with disruption of pancreatic lysosomes and subsequent release of lysozymes and proteases. These substances then supposedly act on certain plasma proteins to release myocardial depressant factor. The author has shown that pancreatectomy and glucocorticoids both tend to lower circulating depressant factor while pancreatitis in non-shocked dogs causes a small increase.

Although no definitive mechanism for the deterioration seen in hemorrhagic shock has yet been discovered, it is apparent that the heart is affected. Whether directly affected by shock per se or secondarily by circulatory changes or other factors, the heart is involved to a significant extent in the homeostatic defenses during hemorrhagic

shock. The reaction of this organ to the stresses of hemorrhage have been extensively studied by many investigators.

Horvath et al.[19] have examined cardiac dynamics and coronary blood flow during shock conditions. As dogs had four per cent of their blood volumes removed within fifteen minutes, their mean arterial pressures fell from one hundred and twenty-seven to thirty-five millimeters of mercury. Cardiac output fell from 2.6 liters per minute to 0.2 liters per minute and coronary vascular resistance decreased from 1.51 to 0.74, resulting in a net decrease of coronary blood flow from eighty-five to forty-eight milliliters per one hundred grams left ventricle per minute. Left ventricular oxygen consumption fell (7.1 milliliters per minute to 4.2 milliliters per minute) but so too did left ventricular work (4.6 kilogram-meters per minute to 0.8 kilogram-meters per minute). At the same time, left ventricular oxygen extraction increased and coronary sinus oxygen content decreased. The authors conclude that dogs in shock are able to compensate for reduced coronary blood flow by increased myocardial oxygen extraction and decreased cardiac work. Opdyke and Foreman[20] measured coronary blood flow following re-infusion of lost blood. They noted up to four times the normal blood flow immediately following transfusion and then a gradual return to normal as coronary artery resistance increased. They believe that there is maximal coronary artery vasodilitation during shock and that

any decrease in coronary blood flow is due solely to decreased blood pressure. Ratliff et al.[21] dispute this finding. Using hyperbaric oxygen, they discovered an inverse relationship between myocardial blood flow and arterial oxygen content in normal dogs. Dogs in shock were found not to have a further reduction in coronary blood flow after the administration of hyperbaric oxygen, but upon going from hyperbaric oxygen to room air the coronary blood flow increased. Thus they conclude that myocardial blood flow in shock is an active process, responding to complex control mechanisms. Bing and Ramos[22] studied the net effect of all these myocardial oxygenation mechanisms by measuring the difference in redox potential between arterial and coronary sinus blood. With a decrease in oxygen, various substances become chemically reduced (DPN to DPN H^+, pyruvate to lactate, etc.) and this forms the basis for changes in redox potential. The difference in redox potential between arterial and coronary sinus blood is normally positive, indicating aerobic metabolism. During oligemic shock these authors found the net redox potential to become even more positive and to fall during irreversible shock but still never become negative. They conclude that there is no myocardial anoxia during oligemic shock and that prevention of anoxia may be due to the intense coronary artery vasodilitation occurring during shock.

Goodyer[23] examined the hemodynamics of the heart during hemorrhagic shock in dogs at a mean arterial blood pressure of forty to fifty millimeters mercury and an oxygen debt of one hundred and forty milliliters per kilogram. He found that the contractile capacity of the myocardium was well maintained or even augmented during the first three hours of shock. Siegel and Downing,[24] however, noted a decrease in both stroke volume and dP/dt during the first two hours of shock in cats at a mean arterial blood pressure of thirty plus or minus five millimeters mercury. Re-infusion of blood at this point did not increase either stroke volume or dP/dt significantly and primarily led to an increased left ventricular end-diastolic pressure, indicating heart failure. Gomez and Hamilton[25] studied left ventricular contractility by observing how well dog hearts could handle an infusion of blood into the left atrial appendage. In normal dogs, there is an immediate increase in cardiac output, increased aortic flow (up to three times the rate of infusion), and a return of left atrial pressure to normal. In dogs bled to a blood pressure of thirty millimeters mercury for ninety minutes and then re-infused to normal pressure, the same manipulation resulted in a normal response initially but after being normotensive for one hundred and fifty minutes there was a marked increase in left atrial pressure while cardiac output remained constant or decreased. The authors concluded that some damage is done to animals in

shock during the hypotensive period which eventually results in decreased myocardial function. Maintaining coronary artery pressure at normal levels during a period of generalized hypotension did not prevent this deterioration. Gauer[26] has described an interesting observation which he feels may account for some myocardial damage during hemorrhagic shock. Normally, the peak left ventricular and aortic pressures are confluent. However, in hemorrhagic shock, while the peak aortic pressure is fifty to sixty millimeters mercury, the peak left ventricular pressure is one hundred and twenty to two hundred millimeters mercury. He concluded that the ventricle is continuing to contract isometrically with considerable force even after its small volume of blood has been expelled.

There is ample evidence of anatomical damage in hearts subjected to hemorrhagic shock. Melcher and Walcott[27] found infiltration of myocardial fibers and actual necrosos of fibers with an accompanying inflammatory reaction following various types of shock in twenty-two out of twenty-nine dogs. The lesions were primarily below the subendocardial surface of the heart and in the papillary muscles. Martin and Hackel[28] bled dogs to a blood pressure of thirty-four millimeters mercury and watched them for two and a half hours before re-infusion. They periodically sacrificed a dog to examine the myocardium. Grossly, there were subendocardial hemorrhages in fourteen out of sixteen dogs that

died or were sacrificed within five days after the shock procedure. Microscopically, there were zonal lesions, described as swollen bands of altered sarcoplasm within the myofibers near intercalated discs, in the subendocardium and papillary muscles in seventeen out of twenty dogs examined as soon as fifteen minutes after shock and up to five weeks after shock. No zonal lesions were seen after five weeks post shock. The zonal lesions were characterized histochemically by depletion of succinic dehydrogenase and cytochrome oxidase activity with adjacent bands of increased enzyme activity. In addition, there were segmented lesions consisting of isolated single cells or groups of cells with coarse granules which stained deeply for succinic dehydrogenase and cytochrome oxidase. Segmental lesions were only seen from nine to forty-eight hours after shock. Finally, advanced lesions of necrotic myofibers plus inflammatory reactions were seen in eight of ten dogs that survived more than fourteen hours following shock. The authors believe that the zonal lesions, appearing very shortly after shock represent reversible early metabolic disruption of the myocardial cell, that segmental lesions represent early cell necrosis and advanced lesions signify an area of extended necrosis. In another study, Martin and Hackel[29] used the electron microscope to further examine these lesions in dogs bled to thirty-five millimeters mercury for only one hour. Under the electron microscope, zonal lesions were marked by hypercontraction with fragmentation of myofibrils and

displacement of mitochondria. All of these lesions affected only a portion of the myocyte (near the intercalated disc). Their appearance within four to eight days was accompanied by relaxation of the hyper-contraction. Areas of necrosis were seen only after six hours and were gone by two to three weeks. The authors could not determine whether areas of necrosis were derived from severe zonal lesions.

Martin et al.[30] viewed the contractions of the heart during hemorrhagic shock via cineangiocardiography. They observed: a) a marked decrease in end-diastolic and end-systolic volumes one hour after shock, b) obliteration of the apical portion of the left ventricular cavity during systole and c) prolonged isometric contraction. Measuring intra-ventricular pressures, they recorded a pressure gradient of one hundred and fifty to three hundred millimeters mercury between the apical and basal portions of the left ventricle. Their conclusion was that physical damage to the myocardium may be a result of abnormal contraction during shock. Hackel et al.[31] extended this theory by examining the lesions of dog hearts that were subjected to heart block during hemorrhagic shock so that the heart rate never exceeded thirty to forty beats per minute. They noted a decrease in both zonal lesions and subendocardial hemorrhage. Ratlif et al.[32] attempted to elucidate the role of hypoxia in the production of these lesions. They subjected dogs to hyperbaric oxygen

during hemorrhagic shock and found significantly less subendocardial hemorrhage (the hemorrhagic lesions were inversely proportional to venous pO_2) but no change in the frequency of zonal lesions. Their conclusion therefore was that hypoxia is responsible for subendocardial hemorrhage and necrosis while zonal lesions are due to other causes (i.e. severe hyper-contraction).

In order to understand the subtle (and perhaps earliest) myocardial derangements during hemorrhagic shock, one must understand the metabolic pathways for energy production normally available to myocardial cells and relate them to alterations produced by shock. Bing[33,34] has found the myocardium to be amazingly versatile in its ability to utilize various substrates for energy production. Glucose, pyruvate, lactate, fatty acids, amino acids, and ketones are all used under normal conditions in the following ratio: glucose 17.9%, pyruvate 0.54%, lactate 16.4% (total carbohydrates 34.9%), fatty acids 67%, amino acids 5.6%, and ketones 4.3% (total non-carbohydrates 76.9%). The rate of utilization of the individual carbohydrates was found to be a function of their arterial concentration and not related to cardiac work. Goodale and Hackel[35] found the coefficient of extraction (arteriovenous difference divided by arterial concentration) to be approximately equal for lactate and pyruvate. Thus, the low arterial concentration of pyruvate accounts for its

small contribution to energy production. While lactate and pyruvate can be extracted from the arterial system at very low concentrations, a minimum concentration of glucose (54.2 mg%) is required. The extractions of glucose, pyruvate and lactate were found to be independent of each other and proportional only to their arterial concentrations. The extraction coefficients appeared not only to be unaffected by cardiac work, but also by coronary artery blood flow. They calculated total carbohydrate utilization under normal conditions to be as high as sixty-six per cent (based on a respiratory quotient of 0.91). At very high concentrations, glucose and pyruvate extraction alone could account for over one hundred per cent of oxygen extraction if totally oxidized. Therefore at high concentrations, some glucose must be stored as glycogen. In later studies Bing et al.[36] suggested that glucose and lactate compete as sources of energy and that an increase in the arterial concentration of one may decrease the extraction of the other.

Although arterial concentration may be the primary determinant of carbohydrate utilization, competition, particularly between carbohydrates and fatty acids, for either extraction or utilization also plays a role. Shipp et al.[37] labeled palmitate (a fatty acid) with C^{14} and noted that when fatty acids are the only substrate available, they are readily removed from the arterial system and approximately fifty per cent is converted to carbon dioxide, the rest to tissue lipids. The addition of glucose into the arterial system had

no effect on fatty acid uptake nor conversion to carbon dioxide. However, when the fate of radioactive glucose was followed with non-radioactive fatty acids present, glucose uptake was slightly decreased, glucose oxidation to carbon dioxide was markedly reduced and radioactive glycogen stores were increased. The same observations were made by Bowman.[38] The conclusions reached were that the heart preferentially utilizes fatty acids as an energy substrate. Evans et al.[39,40] studied the interrelationship of pyruvate and fatty acids. Using labeled pyruvate they found that pyruvate uptake is increased as the arterial concentration of pyruvate increases, but the per cent of available pyruvate that was extracted decreased as concentrations became higher. At each concentration, approximately sixty per cent of the extracted pyruvate was converted to carbon dioxide. At a concentration of five to ten millimoles per liter, pyruvate could account for total carbon dioxide production. The addition of palmitate decreased pyruvate extraction and decreased its oxidation to carbon dioxide even more. The decarboxylation of pyruvate decreased in proportion to the fatty acid concentration while total carbon dioxide production remained the same. At high concentrations (five to ten millimoles per liter) pyruvate did not decrease the uptake of radioactive palmitate but did cause a decrease in the quantity recovered as labeled carbon dioxide and an increase in the amount of labeled tissue lipids. Krasnow et al.[41] found a decreased

lactate uptake during conditions favoring intracellular glycolysis or increased free fatty acid uptake such as mild tachycardia, moderate hypoxia and anemia.

Neely et al.[42] have done an extensive review of the intermediary metabolic pathways and metabolic controls of the heart. They found that in the absence of insulin (required for facilitated glucose transport across the cell membrane) glucose uptake is decreased and the rate of glucose transport into the myocardial cell is the limiting factor in its utilization as an energy source. In the presence of insulin, glucose uptake is increased by cardiac work (contrary to the experience of Bing)[33] and decreased by ketones and fatty acids. Fatty acids, however, do not inhibit glucose uptake in anaerobic hearts, suggesting that they must be metabolized before they can exert an inhibitory effect. Free glucose must be converted to glucose-6-phosphate before it can be further metabolized. The enzyme, hexokinase, which performs this function is inhibited by glucose-6-phosphate, thus forming a feed-back regulatory loop. Glucose-6-phosphate can go into glycogen storage, the glycolytic pathway or the pentose-phosphate shunt. Conversion of fructose-6-phosphate to fructose-1,6-diphosphate by phosphofructokinase is the first irreversible step in the glycolytic pathway (glucose-6-phosphate to fructose-6-fructose-6-phosphate is reversible). Phosphofructokinase is inhibited by ATP and citrate, and enhanced by free phosphate,

5'AMP, ADP and cyclic AMP. Pyruvate dehydrogenase which converts pyruvate (the end product of the glycolytic pathway) to acetyl-CoA is the next major regulatory enzyme and is the common link for the aerobic utilization of all carbohydrate substrates. This enzyme is inhibited by ATP, NADH, and acetyl-CoA. Its activity is enhanced by NAD. In addition to allosteric control, pyruvate dehydrogenase is controlled by phosphorylation (inactive) and dephosphorylation (active) mechanisms similar to those involved in glycogen storage. The preferential use of fatty acids in normal myocardial metabolism involves inhibition of hexokinase, phosphofructokinase and pyruvate dehydrogenase via acetyl-CoA, NADH, ATP and citrate accumulations plus stimulation of glycogen synthetase and inhibition of glycogen phosphorylase which are involved in glycogen storage pathways.

The rate of free fatty acid uptake by the heart is proportional to the serum concentration, but levels off at very high concentrations. Increased albumin concentration and reduced oxygen supply will inhibit uptake. Utilization of fatty acids is primarily determined by conversion of acyl-CoA to acetyl-CoA via beta oxidation. Beta oxidation is inhibited by accumulation of acetyl-CoA and decreased oxidative phosphorylation. Another limitation to the utilization of fatty acids is due to the prevention of translocation across the inner mitochondrial membrane of acyl-carnitine

units which occurs with increased cardiac work either because of reduced availability of free CoASH (used also in the succinic dehydrogenase reaction of the TCA cycle) or decreased formation of transferase II.

The TCA cycle is regulated by ATP/ADP and NADH/NAD ratios (increases as the ratio decreases) plus the actions of certain regulatory enzymes. Citrate synthetase activity depends on the availability of acetyl-CoA and oxaloacetate and is inhibited by free CoASH and succinyl-CoA. NAD specific isocitrate dehydrogenase is activated by ADP and inhibited by NADH and ATP. Finally, alpha-ketoglutarate dehydrogenase is inhibited by succinyl-CoA and NADH.

We can now turn to what is known of myocardial metabolism during hemorrhagic shock and see how it compares with metabolism under normal conditions. Spitzer and Spitzer[43] used labeled free fatty acids during early hemorrhagic shock (prior to anaerobic metabolism) to determine the relative utilization of fatty acids versus carbohydrates. In control dogs there was considerable fatty acid and lactate extraction plus glucose extraction in less than half. Myocardial carbon dioxide production was two-thirds free fatty acid and approximately one-third lactate derived. During early hemorrhagic shock, free fatty acid extraction decreased, lactate extraction remained at its normally high level and glucose extraction was found in all dogs. Myocardial carbon

dioxide production shifted to only one fifth derived from fatty acids and approximately two thirds from lactate. Thus in early hemorrhagic shock, dog myocardiums rely more on carbohydrate metabolism than fatty acid metabolism. Additionally, Doersching and Glaviano[44] found that although the total concentration of phosphorylase remained constant during shock, the activity of phosphorylase a (which leads to increased glycogen production) is increased. The actual concentration of glycogen within the myocardium was also measured and found to be increased. Hence significant glycolysis is not present during hemorrhagic shock as a source of energy substrates. This leaves exogenous carbohydrates as the major energy source during hemorrhagic shock.

According to Hackel and Goodale[45] arterial pyruvate concentration increases during hemorrhagic shock but the arterio-venous difference does not increase and eventually becomes negative. Hence, impaired pyruvate metabolism is implied. Arterial lactate concentration increases and the arteriovenous difference becomes slightly more positive. However, the increase in uptake is less than the increase in arterial concentration so that the coefficient of extraction decreases. Arterial glucose concentration also increases but glucose extraction remains constant and eventually decreases (never becomes negative). Despite the low blood pressure and cardiac output, coronary flow did not appear to decrease. Due to tachycardia and increased myocardial

oxygen extraction (from sixty-four to eighty-five per cent) total utilization of oxygen by the myocardium remained normal. The authors found the negligible or negative pyruvate extraction to be the most consistent and striking abnormality in hemorrhagic shock. The effect of high arterial lactate concentration in diminishing the uptake of pyruvate was disproved by infusing lactate into normal dogs and noting no change in pyruvate extraction. Burdette[46] found a high pyruvate content in cardiac muscle cells in hemorrhagic shocked dogs and concluded that this may represent a breakdown in the ability of carbohydrates to enter the TCA cycle. This was observed in early hemorrhagic shock before the presence of lactic acid production. Marchetti et al.[47] observed that pyruvate was normally discharged from the myocardiums of unanesthetized dogs but that this negative extraction becomes even greater during shock. There was no change in myocardial oxygen consumption until the dogs' blood pressures fell below seventy to eighty millimeters mercury (when it fell to fifty-seven per cent of normal). However, at this point the cardiac work fell to ten per cent of normal. The redox potential across the heart remained positive and there was no excess lactate. The authors suggest that myocardial damage during early shock is represented by the increased pyruvate discharge and that this is not due to hypoxia but may be the result of excessive catecholamines or peripheral factors. Burdette et al.[48,49] noted that oxygen consumption decreased

after four hours in rats kept in hemorrhagic shock. The oxygen consumption could be increased by pyruvate infusions. Therefore, they concluded that animals in shock are still able to metabolize pyruvate. They noted, however, that the increase in oxygen consumption is not as great as that found in normal dogs given the same amount of pyruvate.

Edwards et al.[50] ascribe the negative pyruvate extraction to hypoxic destruction of cocarboxylase (thiamine pyrophosphate), the coenzyme needed to convert pyruvate to acetyl-CoA. They claim that in hemorrhagic shock the coronary blood flow decreases while the per cent oxygen extraction remains constant, hence a relative ischemic condition. They explain the continued utilization of lactate during negative pyruvate extraction (and a presumed block at the cocarboxylase level) on either conversion to glycogen via glycolysis reversal or direct oxidation via lactic acid oxidase I (requiring only lactate, molecular oxygen and cytochrome c). Hackel and Bretenecker[51] studied the time factor in the production of metabolic derangement during hemorrhagic shock by comparing dogs bled to thirty-two millimeters mercury for three to three and a half hours with those in shock for only one to one and a quarter hours. The pyruvate extraction became increasingly lower and finally negative as shock persisted. Transfusions after one hour restored normal pyruvate metabolism while after three hours it could not. They concluded that progressive

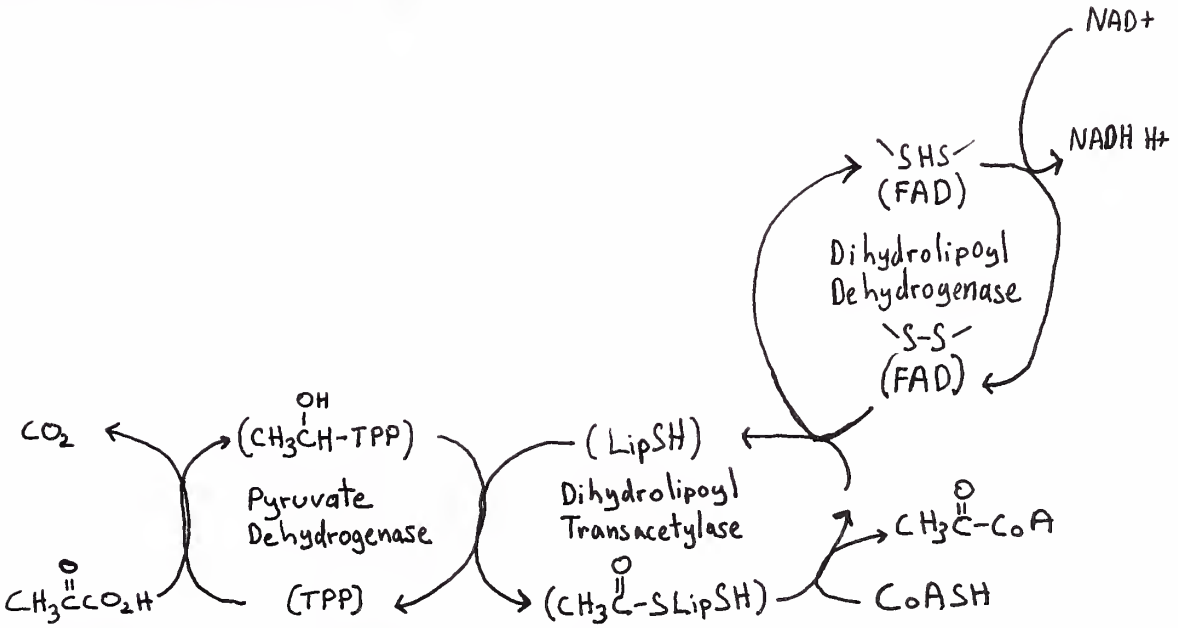
ischemia leads to irreversible enzyme changes. Furthermore, the localization of the defect to cocarboxylase was reenforced by noting that the myocardial oxygen extraction was proportional to the inverse of the coronary blood flow which they claim is seen in thiamine (cocarboxylase substrate) deficiency and not in normal dogs.

McKeever et al.[52,53] maintained the circulation of the coronary vessels at normal levels while placing dogs in hemorrhagic shock and still noted abnormal pyruvate metabolism and eventually irreversible shock. Hence, they believe myocardial damage can occur during shock despite maintenance of adequate coronary flow and oxygen availability. Ratliff et al.[54] were able to increase a decreased lactate extraction, which they found during hemorrhagic shock, by using oxygen at a high pressure in their experiments. They were not able to correct pyruvate metabolism or prevent zonal lesions. Goodyer et al.[55] bled dogs to an aortic pressure of thirty to forty millimeters mercury and then: a) used an inflatable balloon in the aorta to keep coronary blood pressure at ninety to one hundred and thirty millimeters mercury, b) cross-transfused a shock dog to a normotensive dog and c) used a pump between the right atrium and femoral artery to sustain systemic pressure at eighty to one hundred and twenty-five millimeters mercury while coronary artery pressure remained at thirty to forty millimeters mercury due to a ligature around the aorta. The balloon support of

coronary artery pressure failed to prevent negative pyruvate extraction but could not normalize it. Support of the systemic pressure prevented negative pyruvate extraction for sixty to seventy minutes. Their conclusion was that the abnormal myocardial production of pyruvate is not due simply to reduced coronary artery perfusion but is related to a peripheral factor which can be partly corrected by maintaining normal systemic blood flow. Landsgaard-Hansen et al.[56,57] measured the activities of various enzymes, glucose-6-phosphate dehydrogenase, pyruvate kinase and lactate dehydrogenase in different parts of the left ventricle (from the apex to base). In normal dogs there was no change in activity of these enzymes from one position of the ventricle to another, but during hemorrhagic shock there were gradients of enzyme activity (activity greater in apical regions than basal). Gradients were also discovered during myocardial hypoxia but they followed a different distribution. Owing to the ability of beta-blockers to abolish the abnormal shock gradients, they concluded that excessive catecholamine activity may be responsible for myocardial damage via excessive contractions.

The most commonly noted early metabolic derangement of hemorrhagic shock is negative myocardial pyruvate extraction. A likely reason for this would be a metabolic block which permitted carbohydrate metabolism to proceed to the stage of pyruvate production but prevented subsequent

introduction of pyruvate into the TCA cycle. The pyruvate dehydrogenase complex is responsible for conversion of pyruvate to acetyl-CoA and hence bridges the gap between the glycolytic and TCA cycles. The complex consists of three enzymes in non-covalent linkage, pyruvate dehydrogenase, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase. Their activity depends on the presence of a flavoprotein, thiamine pyrophosphate (cocarboxylase) and NAD as diagramed below:[58]



The complex has a molecular weight of four million and is alkaline sensitive. The activity of the complex is regulated by a phosphorylation (inactive) and de-phosphorylation (active) system at the pyruvate dehydrogenase step.[59] The phosphotase enzyme requires ten times as much magnesium ion as the kinase enzyme. Therefore, the activity of the complex is greater when the ATP/ADP ratio is low and consequently

more free magnesium is available. The complex is also inhibited by acetyl-CoA acting on pyruvate dehydrogenase and NADH acting on the flavoprotein.[60] Fatty acids inhibit the complex via: a) acetyl-CoA and NADH production, b) competition of enzyme systems for CoA and NAD, c) direct effect of pyruvate dehydrogenase and d) ATP production. Thiamine pyrophosphate, (cocarboxylase), is broken down when oxygen is withdrawn in vitro and is split by a phosphotase under conditions of anoxia in tissue extracts.[61]

The following experiments attempt to examine this critical step in pyruvate (and carbohydrate) metabolism during hemorrhagic shock by determining the effects of thiamine on the abnormal myocardial pyruvate extraction seen during shock and by assaying the activity of both the pyruvate dehydrogenase complex and cocarboxylase in normal and shocked dogs.

METHODS AND MATERIALS

Experiment I

Ten mongrel dogs (21.5 - 43.0 lbs.) were anesthetized intravenously with allobarbital .44 cc/kg (Dial with Urethane, CIBA Pharmaceutical Co.). A number 7F NIH catheter (U. S. Catheter and Instrument Co.) was inserted into the right external jugular vein. PE 330, 0.147" OD polyethylene tubing (Clay Adams) attached to a three-way stopcock was used for cannulating the right carotid artery. The left femoral artery and vein were cannulated with number 7 courand catheters (U. S. Catheter and Instrument Co.). All catheters were flushed with a solution of 1 cc heparin in 500 cc normal saline. After catheterization, the dogs were intubated and respiration was maintained via a compressed air ventilator.

Under flouroscopy, the right external jugular catheter was positioned in the coronary sinus. Two cc of blood were slowly withdrawn to determine per cent oxygen saturation. Normal venous blood is approximately 60 to 80% oxygen saturated. Coronary sinus blood, due to high myocardial oxygen extraction, is normally only 20 to 40% saturated. Finding a low per cent saturation in blood obtained from the positioned catheter was used as evidence of proper placement.

At autopsy the correct position of the catheter tip was again verified.

The dogs were then heparinized with sodium heparin .15 cc/kg intravenously. Arterial blood pressure was measured and arterial and coronary sinus blood samples were drawn prior to placing the dogs in hemorrhagic shock. Blood pressure was determined by a transducer connected to the femoral artery catheter and a photorecording oscilloscope (Electronics for Medicine, White Plains, N. Y.). The transducer was calibrated at several pressures with a mercury column prior to each experiment. Arterial and coronary sinus blood samples were analyzed for hemoglobin content, per cent oxygen saturation, pyruvate and lactate concentrations. Before obtaining blood samples, the catheters were flushed by withdrawing and discarding ten milliliters of blood.

Oxygen saturation readings were done on 1-2 cc of unhemolyzed blood with the Reflection Oximeter (American Optical). Hemoglobin content was measured on 20 cu mm blood samples with a Fischer Hemophotometer. pH determinations were performed on selected samples with a Model 113 pH and blood gas analyzing system, employing glass electrodes to measure the potential established between unknown and standard reference solutions.

Lactate and pyruvate assays were performed on a Teco-meter (Boehringer Mannheim Corp.) using Teco-meter kits.

The assays are based on the reaction:

$$\text{pyruvate} + \text{NADH H+} \overset{\text{LDH}}{\rightleftharpoons} \text{lactate} + \text{NAD+}$$

A surplus of NADH plus LDH will shift the reaction to the right while NAD will do the opposite. The amount of shift depends upon the concentration of pyruvate in the former case and lactate in the latter. Since NADH absorbs light at a peak wavelength of 340nm and NAD does not absorb between 300-400nm, optical densities can be used to determine the extent of the reactions and thus the substrate concentrations.

To determine lactate concentration, 4cc of iced 0.6N perchloric acid (a protein denaturant) was pipetted into a test tube. Both tube and acid were weighed together. A 2cc blood sample was added and the tube reweighed to determine net blood weight. The solution was then centrifuged at 3000 rpm for 30 minutes. 0.20ml of supernatant was added to a cuvette containing 2.0ml of 0.5M glycine buffer (pH 9.0), 0.20ml NAD (27mM/7ml), and 0.02ml LDH (2mg/ml). The optical density of this solution was read at 366 nm wavelength. A blank was made by adding 2.0ml of 0.5M glycine buffer, 0.20ml perchloric acid (0.6N), 0.20ml NAD (27mM/7ml), and 0.02ml LDH (2mg/ml). The difference in optical density between the sample and the blank times calibration and weight correction factors gives the final lactate concentration in mg/100ml blood.

$$\text{lactate (mg/100ml)} = 94.1 \times \frac{4+\text{blood wt.}}{4\times\text{blood wt.}} \times (\text{O.D. sample} - \text{O.D. blank})$$

For pyruvate determinations, 11ml of 60% perchloric solution was added to 89ml of iced distilled water and weighed. To this solution (approximately 1N perchloric acid) an 8ml blood sample was added. The final solution was weighed and then centrifuged at 3000 rpm for 30 minutes. Four ml of supernatant was mixed with 2.0ml of a 0.7M tri-potassium phosphate buffer. The buffered sample was kept on ice for ten minutes, filtered and then warmed in a 25° centigrade bath for one hour. Afterwards, 2.0ml of filtrate was combined with 0.2ml of NADH (2.5mM/4ml). The optical density of this solution was read at 366 nm wavelength. With the addition of 0.02ml of LDH (2mg/ml) the optical density was again recorded. The change in optical density multiplied by calibration and weight factors gives the pyruvate concentration in micro-moles/ml blood.

$$\text{pyruvate (micro-moles/ml)} = \frac{8.21}{9.2} \times \frac{8+\text{blood wt.}}{2\times\text{blood wt.}} \times \text{delta O.D.}$$

Hemorrhage was begun by attaching a glass bottle and a 50 cc heparinized syringe to the three-way stopcock on the carotid artery cannula. The dogs were bled by letting their blood flow freely into the glass bottle until the mean arterial pressure was between 30-40mm Hg. Bleeding time averaged approximately five minutes. Periodically, blood was either withdrawn or injected via the 50cc syringe in order to maintain a constant blood pressure.

During shock, the following procedures were followed:

Chart 1

Procedure for Experiment I

Dog	Shock +60 min.	+90 min.	+120 min.	+150 min.	+180 min.	+210 min.	+240 min.
1	art.+c.s. blood sample -then- 5cc (500mg) thiamine hydro- chloride (Lilly)		art.+c.s. blood sample -then- 5cc (500mg) thiamine hydro- chloride			art.+c.s. blood sample -then- blood returned	art.+c.s. blood sample
2	art.+c.s. blood sample -then- 5cc (500mg) thiamine hydro- chloride	art.+c.s. blood sample -then- 5cc (500mg) thiamine hydro- chloride		art.+c.s. blood sample -then- blood returned	art.+c.s. blood sample		
3	art.+c.s. blood sample -then- 5cc (500mg) thiamine hydro- chloride	art.+c.s. blood sample -then- 5cc (500mg) thiamine hydro- chloride		art.+c.s. blood sample -then- blood returned	art.+c.s. blood sample		

Chart 1 (cont'd.)

Dog	Shock +60 min.	+90 min.	+120 min.	+150 min.	+180 min.	+210 min.	+240 min.
4	art.+c.s. blood sample -then- 10cc (1000mg) cocarboxy-lase (Sigma)	art.+c.s. blood sample -then- 10cc (1000mg) cocarboxy-lase	art.+c.s. blood sample	art.+c.s. blood sample -then- blood returned	art.+c.s. blood sample		
5	art.+c.s. blood sample -then- 25cc (2500mg) cocarbosy-lase	art.+c.s. blood sample -then- 25cc (2500mg) cocarboxy-lase	art.+c.s. blood sample -then- 20cc (2000mg) cocarboxy-lase	art.+c.s. blood sample	art.+c.s. blood sample -then- blood returned	art.+c.s. blood sample	
6	art.+c.s. blood sample -then- 1120cc 6% dextran in normal saline (Cutter)	art.+c.s. blood sample -then- 200cc 6% dextran in normal saline	art.+c.s. blood sample -then- 1000cc blood removed	500cc blood returned -then- art.+c.s. blood sample	art.+c.s. blood sample	art.+c.s. blood sample	

Chart 1 (cont'd.)

Dog	Shock +60 min.	+90 min.	+120 min.	+150 min.	+180 min.	+210 min.	+240 min.
7	art.+c.s. blood sample -then- 750cc 6% dextran in normal saline	art.+c.s. blood sample	art.+c.s. blood sample	art.+c.s. blood sample	art.+c.s. blood sample		
8	art.+c.s. blood sample -then- 600cc 6% dextran in normal saline	art.+c.s. blood sample	art.+c.s. blood sample	art.+c.s. blood sample	art.+c.s. blood sample	art.+c.s. blood sample	
9	art.+c.s. blood sample -then- 50ml $NaHCO_3$ (375gm, 44.6 mEq) (Invenex) + 10cc (1gm) thiamine hydrochloride	art.+c.s. blood sample		art.+c.s. blood sample		art.+c.s. blood sample	

Chart 1 (cont'd.)

Dog	Shock +60 min.	+90 min.	+120 min.	+150 min.	+180 min.	+210 min.	+240 min.
10	art.+c.s. blood sample -then- 900cc 6% dextran in normal saline	art.+c.s. blood sample	art.+c.s. blood sample		art.+c.s. blood sample		

In all cases, arterial and coronary sinus samples were taken before shock, one hour after induction of shock and 30 minutes after re-infusion of shed blood. Blood pressures were continuously monitored. Thiamine hydrochloride, cocarboxylase and sodium bicarbonate were injected into the left femoral vein. Dextran in normal saline was administered directly into the right carotid artery.

Experiment II

Fourteen mongrel dogs (29.0 - 44 lbs) were anesthetized with thiamylal (Surital, Park Davis), 1cc/5lbs., intravenously. Seven were cannulated and shocked as in experiment I. Arterial and coronary sinus blood samples were analyzed for pyruvate, lactate and per cent oxygen saturation just before and one hour after shock. After obtaining the second blood sample, a surgical non-traumatic clamp was placed across the heart and apical and basal left ventricular tissue samples (approximately one gram each) were removed from the left ventricle. The samples were assayed for pyruvate dehydrogenase and cocarboxylase activity. Seven dogs served as controls. They were cannulated and intubated similarly to the shock dogs. Arterial and coronary sinus blood samples were obtained and then a small piece of left ventricle apex was removed. Bleeding was prevented by keeping a surgical non-traumatic clamp across the heart. The dogs were kept

on a respirator for one hour. Then a small basal piece of left ventricle was removed.

The pyruvate dehydrogenase assay was based on the work of Gubler[62] and Cooper.[63] As pyruvate is chemically oxidized to acetyl-CoA via a complex series of enzymatic steps, pyruvate loses one carbon dioxide molecule and one hydrogen ion. The oxidation of pyruvate can be coupled with the reduction of ferric cyanide to ferrous cyanide by providing ferric cyanide as a reactant instead of CoASH. The change from the ferric to ferrous form of iron can be detected by a decrease in optical density at 420 mu wavelength. Thus the activity of the pyruvate dehydrogenase complex can be monitored. By adding cocarboxylase to one cuvette, any decrease in activity due to lack of this coenzyme could also be determined.

Assay solutions were made by a combination of the following chemicals:

Potassium phosphate (1mole/liter) (Fisher)	.20ml
Magnesium chloride (1mole/liter) (Fisher)	.02ml
Sodium pyruvate (1mole/liter) (K & K Lab.)	.08ml
Sodium malate (0.5mole/liter) (K & K Lab.)	.02ml
Thiamine pyrophosphate (.00217mole/liter) Cocarboxylase (Cal Biochem.)	.05ml
Nicotinamide adenine diphosphate (.01mole/liter) (K & K Lab.)	.12ml
Adenoside tri-phosphate (.01mole/liter) (K & K Lab., P & L Biochem., Worthington)	.80ml
Distilled Water	1.05ml

Solution one contained everything except sodium pyruvate and sodium malate as a control. Solution two contained everything except thiamine pyrophosphate (cocarboxylase) in order

to determine cocarboxylase activity. Solution three contained all ingredients and served as an assay for pyruvate dehydrogenase activity.

0.5 grams of tissue were added to 4.5ml of .32M sucrose (maintaining a 1:9 ratio). The tissue solution was homogenized in a Tri-R Stir-R ground glass homogenizer (Model R41, Rockville Center, N. Y.) until there were no lumps remaining (approximately five minutes). The homogenized solution was centrifuged at 1600 rpm for 15 minutes to remove nuclear debris. The supernatant was poured into a fresh test tube kept in an ice bucket.

A Beckman DU spectrophotometer set at 420 mu was used to record optical densities. All readings were taken with solutions at room temperature. Originally solution one plus 0.5ml of homogenate supernatant was used to zero the machine. However, the addition of 3.6ml of sodium ferricyanide .01mole/liter (Fisher), which is needed to start the reaction, would cause readings to begin in optical density ranges which resulted in either the absorbancy needle going off scale during the 20 minute reaction period or produce absorbancy readings in an area of the scale which was too grossly calibrated to provide accurate readings. Therefore, the homogenate supernatant and ferricyanide were added to all three solutions at the same time and the machine was zeroed so that total solution number one fell at an absorbancy of 0.800 by adjusting the light slit. To reduce any

major differences in turbidity due to the dense homogenate, all cuvettes were read through the frosted side (as per Gubler and Cooper). Since all three total solutions were originally of approximately the same optical density, all three total solutions were found to begin at approximately 0.800 absorbancy. Immediately after the machine was zeroed, the optical densities of total solutions one, two, and three were read and subsequently recorded every minute thereafter for 20 minutes.

The control solution (number one) changed slightly in optical density over the 20 minute reaction period. This is probably due to some endogenous substrate or reducing agents contained within the homogenate supernatant.

The changes in absorbancy per minute for all three solutions were calculated and the control changes were subtracted from each of the experimental samples. The net absorbancy changes were plotted on graph paper and the slope, delta A/20 minutes, was determined. Except for early erratic recordings (probably due to endogenous oxidizing and reducing agents) and a final tapering (probably due to exhaustion of substrate) a linear relationship between absorbancy and time was noted. Unfortunately, the activity of the control samples was so great that only a few readings could be taken before the absorbancy needle went off scale (even when starting at .800A). A sample of the homogenate

supernatant was sent to the Yale-New Haven Hospital Clinical Laboratories for nitrogen content analysis. The final enzyme and coenzyme activity was expressed as deltaA/20minutes/mg nitrogen.

Experiment III

Twenty dogs (28-45 lbs.) were anesthetized as per experiment II. Ten dogs were put into hemorrhagic shock as described previously. Apical and basal left ventricular samples were obtained for pyruvate dehydrogenase activity determinations. Arterial and coronary sinus blood samples were obtained before and after one hour of shock and then assayed for pyruvate, lactate and per cent oxygen saturation. Ten dogs served as controls. Apical and basal left ventricular samples were obtained only after one hour on a respirator. Blood samples were drawn before and after one hour on the respirator.

Three major changes in the assay procedure were employed:

a) A Beckman DBG model spectrophotometer was used. This machine automatically zeroes samples of different optical densities and records only the net differences in optical density between two samples after zero time (i.e. while the reaction is progressing). The total assay solutions minus ferricyanide (which starts the reaction) were used to zero the machine.

b) After zeroing the machine, ferricyanide was injected by using tuberculin syringes. This enabled all reactions to begin virtually simultaneously.

c) Only 0.1ml of homogenate supernatant was used. This decreased the original turbidity enough so that the clear sides of the cuvettes could be employed and also permitted full 20 minute observations of control enzyme activity without going off scale.

Net changes in optical density were read from the spectrophotometer each minute for 20 minutes. The plot of absorbancy versus time was similar to previous graphs. It was discovered that the most consistently linear portion of the graph was between five and ten minutes. This region represents the period after extraneous reducing agents have reacted and before the substrates begin to be critically used up.

All samples were assayed in triplicate and the final enzyme activity was recorded as delta A/5 minutes/mg nitrogen.

RESULTS

Certain generalizations can be made of all dogs placed into hemorrhagic shock. Initial mean arterial blood pressures ranged from 75-150 millimeters mercury (depending to some extent on the level of anesthesia). Approximately 600cc of blood were removed from most dogs, dropping the blood pressures to a range of 25-45 millimeters mercury. At first, the blood pressures tended to return towards normal due to intense vasoconstriction. After 30 minutes and slightly more hemorrhage, the blood pressures stabilized at shock levels. Occasionally, small (25-50cc) infusions of shed blood were required to maintain a steady blood pressure. Coronary sinus oxygen saturation was originally found to lie between 25-50 per cent. During shock it fell by approximately 20-50 per cent in most cases (see Appendix).

Experiment I (See Table 1)

Out of a series of ten dogs, eight showed a change from positive to negative myocardial pyruvate extraction after one hour of hemorrhagic shock. In the two cases that did not show this change, it is noteworthy that the arterial lactate concentrations did not increase as one would expect in shock. Therefore, it may be that these dogs did not go into the same degree of shock as the other dogs, despite similar blood loss. At the same time that the eight

dogs began discharging pyruvate, all but one maintained a positive lactate arteriovenous difference.

When blood was re-infused to five of the eight shocked dogs with negative pyruvate extractions, two showed a return to normal (positive) pyruvate extraction within 30 minutes. The shock blood pressures of the five dogs were all similar but the amount of time spent in shock was slightly less for the two dogs returning to normal (approximately three hours versus three and one half to four hours).

A total of one gram of thiamine hydrochloride was given to three shock dogs with negative pyruvate extractions. In one case 44.6 millequivalents of sodium bicarbonate was also given to neutralize the acidic pH caused by shock. Observations lasting from 30 to 90 minutes revealed no change in the abnormal pyruvate extraction except for two separate single observations which reverted back to abnormality by the next observation.

One dog given two grams of cocarboxylase and one given seven grams both showed persistent negative pyruvate extraction. Since cocarboxylase is the active form of thiamine in the body (thiamine pyrophosphate), its failure to correct the abnormal pyruvate extraction is further evidence of the inability of thiamine administration to significantly improve the abnormal pyruvate metabolism.

Six per cent dextran in normal saline was given in order to restore blood pressure (and intravascular volume) to three dogs with negative pyruvate extraction. With the dextran, blood pressures returned to within 25 millimeters mercury of the original starting blood pressures. Pyruvate metabolism became normalized for at least one observation in each dog. In two cases, when the dextran was given after one hour of shock and then nothing else was done to the dogs, negative pyruvate extraction became positive and remained so for two hours (except for one negative observation in one of them).

The improvement with dextran did not correlate with pH changes. The return to normal pyruvate extraction occurred during alkalotic and acidotic conditions. Hemoglobin concentration and therefore oxygen carrying capacity per millileter of blood decreased with the dextran infusion. However, increased flow probably compensated for this as evidenced by increased coronary sinus oxygen saturations of approximately 20%.

Experiment II (See Table 2, Figures 1,2)

Using the same pyruvate assay procedure as in experiment I, all dogs, control and shock, were found to have negative pyruvate extractions at the start of this experiment. Excluding two dogs, one with negative lactate extraction and one that died during the experiment, the

pyruvate extractions of the shocked dogs became even more negative during shock. The average pyruvate extraction of the shocked dogs before shock was -0.056um/ml while after shock it decreased to -0.135um/ml. The lactate extraction remained positive and increased from an average of 3.38mg% to 11.60mg% during shock. The change in pyruvate extraction was found to be statistically significant, $p<.01$.

The pyruvate dehydrogenase activity of seven control dogs without thiamine pyrophosphate averaged .856±302 A/20min./mg N while five shock dogs averaged only .371±094. This difference was found to be statistically significant with probability <.01. The addition of thiamine pyrophosphate to the assay resulted in an average shock enzyme activity of .406±.085 A/20min./mg N and a control value of .879±.284. Once again, the difference between control and shock samples was found to be statistically significant, probability <.01.

Looking at just the control dogs in regard to changes in enzyme activity brought about by the addition of thiamine pyrophosphate, it appears that there was virtually no change (.856±.302 without and .879±.284 with). In the shock dogs, the addition of thiamine pyrophosphate resulted in an increase in activity from .371±.094 to .419±.091, but this changes is not statistically significant, probability ≥.05.

Keeping the dogs on a respirator for one hour also did not seem to affect enzyme activity to a statistically significant degree. Controls with thiamine pyrophosphate averaged .923±.346 A/20min./mg N before and .834±.195 A/20min./mg N after one hour on the respirator. Similar controls without thiamine pyrophosphate averaged .919±.372 A/20min./mg N and .793±.188 A/20min./mg N, before and after respectively. In both cases a paired t test revealed a probability $\geq .05$ (not significant).

Finally, the enzyme activity in apical and basal samples were compared with each other. In the control group, the average apex value was .921±.359 A/20min./mg N and the basal left ventricular value .814±.193 A/20min./mg N. In the shocked dogs, the apical value was also somewhat greater, .393±.072 A/20min./mg N compared to .358±.105 A/20min./mg N. These differences were not found to be statistically significant using a paired t test.

Experiment III (See Table 3, Figures 3,4)

Ten control dogs all exhibited negative pyruvate extractions (mean -0.0384 um/ml) and positive lactate extractions mean (12.05mg%) at the start of the experiment as in experiment II. After one hour on a respirator there was no change in either pyruvate or lactate extraction (-0.0380um/ml and 11.66mg% respectively, $p \geq .05$).

Of the ten shock dogs, three developed negative lactate extraction after shock and will not be discussed further. The remaining seven had an original pyruvate extraction of -0.343um/ml, which did not differ from the control dogs. After shock, the pyruvate extraction became more negative (mean -0.164um/ml). A paired t test shows this difference to be statistically significant, probability <.02. Lactate extraction was originally at a mean value of 9.85mg% and increased slightly to 14.90mg% during shock. The lactate extraction did not increase in every case and the differences were found not to be statistically significant.

A comparison of the pyruvate dehydrogenase activity in left ventricular base samples between ten control and seven shock dogs revealed a mean of .431±.096 A/5min./mg N for the controls and .321±.086 A/5min./mg N for the shocked dogs. This was found to be statistically significant, probability <.05. Five control base samples without thiamine pyrophosphate had a mean enzyme activity of .453±.144 A/5min./mg N while that of comparable shock samples was .273±.080 A/5min./mg N. This difference was not statistically significant, but this may be due to the small sampling size.

The mean enzyme activity of the shocked dogs' apical samples, .363±.128 A/5min./mg N was not significantly different from corresponding base samples, .393±.111 A/5min./mg N. Control apical samples also did not differ statistically

from base samples in enzyme activity (.397±.095 and .438±.111 respectively). In both groups however, base samples showed slightly greater activity.

No effect was seen in regard to enzyme activity by the addition of thiamine pyrophosphate to shocked dogs' left ventricular base assays. The mean value without thiamine pyrophosphate was .273±.080 A/5min./mg N while with it it was .292±.085 A/5min./mg N. In control dogs too there was no difference. A mean of .423±.078 A/5min./mg N was obtained without thiamine pyrophosphate and .453±.144 A/5min./mg N with it (probability >.05).

TABLE 1

Results of Experiment I

Dog	A-V Diff. Before Shock Pyr. um/ml	A-V Diff. Before Shock Lact. mg%	A-V Diff. After Shock Pyr. um/ml	A-V Diff. After Shock Lact. mg%	Therap. Agent	A-V Diff. With Therap. Pyr. um/ml	A-V Diff. With Therap. Lact. mg%	Approx. Time After Start of Therap.	A-V Diff. Blood Ret. Pyr. um/ml	A-V Diff. Blood Ret. Lact. mg%
1	0.132	11.550	-0.005	4.512	Thiamine HCl	-0.039	14.746	1 hr.	-0.124	
						0.040	18.247	2½ hrs.		
2	0.062	9.668	0.031	10.193	Thiamine HCl	0.038	8.832	½ hr.	-0.049	9.753
						0.052	10.798	1½ hrs.		
3	0.033	6.873		13.113	Thiamine HCl	-0.022	11.350	½ hr.	0.114	15.005
						-0.084	14.006	1½ hrs.		
9	0.041	8.497	-0.830	11.562	Thiamine HCl	-0.063	11.339	½ hr.		
						0.001	14.206	1½ hrs.		
					Sodium Bicarbonate	-0.044	15.264	2½ hrs.		
4	0.042	-0.376	-0.096	9.143	Cocarboxylase	-0.100	16.802	½ hr.	0.002	18.612
						-0.101	11.480	1 hr.		
						-0.027	11.115	1½ hrs.		
5	0.007	10.510	-0.136	3.315	Cocarboxylase	-0.112	10.387	½ hr.	-0.053	7.414
						-0.047	8.437	1 hr.		
						-0.029	3.459	1½ hrs.		
						-0.040	9.821	2½ hrs.		

TABLE 1 (Cont'd.)

Dog	A-V Diff. Before Shock Pyr. um/ml	Lact. mg%	A-V Diff. After Shock Pyr. um/ml	Lact. mg%	Therap. Agent	A-V Diff. With Therap. Pyr. um/ml	Lact. mg%	Approx. Time After Start of Therap.	A-V Diff. Blood Ret. Pyr. um/ml	Lact. mg%
6	0.181	11.480	-0.095	6.792	Dextran	-0.011	1.750	½ hr.	-0.061	7.285
						0.006	-0.179	1 hr.		
7	0.258	10.763	-0.056	-2.420	Dextran	0.046	0.769	½ hr.		
						0.006	-2.033	1 hr.		
						-0.116	-1.480	1½ hrs.		
						0.011	-2.033	2 hrs.		
8		18.295	0.013	12.443	Dextran	0.402	7.544	½ hr.		
						0.045	8.813	1 hr.		
						0.006	6.827	1½ hrs.		
						0.007	5.416	2 hrs.		
						0.025	6.827	2½ hrs.		
10	0.071	18.095	-0.044	10.298	Dextran	0.003	-12.020	½ hr.		
						0.316	7.838	1 hr.		
						0.307	4.441	2 hrs.		

TABLE 2

Results of Experiment II

Subject	Mean±SD	Subject	Mean±SD	t test	Probability
		A-VPyruvates (um/ml)			
Before Shock N=5	-0.056±.045	After Shock N=5	-0.135±.049	2.95 (paired)	<.01
		A-V Lactates (mg%)			
Before Shock N=5	3.38±3.60	After Shock N=5	11.60±4.59	2.88 (paired)	N.S.* ≥.05
	Pyruvate Dehydrogenase Complex Enzyme Activity (A/20min./mg N) With Thiamine Pyrophosphate				
Control Dogs N=7	.879±.284	Shock Dogs N=5	.406±.085	4.64	<.01
	Pyruvate Dehydrogenase Complex Enzyme Activity (A/20min./mg N) Without Thiamine Pyrophosphate				
Control Dogs N=7	.856±.302	Shock Dogs N=5	.302±.094	4.72	<.01
	Control Dogs' Enzyme Activity (A/20min./mg N)				
With Thiamine Pyrophosphate N=7	.879±.284	Without Thiamine Pyrophosphate N=7	.856±.302	1.01 (paired)	N.S. ≥.05

*N.S. = not significant

TABLE 2 (Cont'd.)

Subject	Mean±SD	Subject	Mean±SD	t test	Probability
	Shock Dogs' Enzyme Activity (A/20min./mg N)				
With Thiamine Pyrophosphate N=5	.419±.091	Without Thiamine Pyrophosphate N=5	.371±.094	1.31 (paired)	N.S. >.05
	Control Dogs' Enzyme Activity (A/20min./mg N) With Thiamine Pyrophosphate				
Before One Hour on Respirator N=7	.923±.346	After One Hour On Respirator N=7	.834±.195	1.35 (paired)	N.S. >.05
	Control Dogs' Enzyme Activity (A/20min./mg N) Without Thiamine Pyrophosphate				
Before One Hour On Respirator N=7	.919±.372	After One Hour on Respirator N=7	.793±.188	1.26 (paired)	N.S. >.05
	Regional Enzyme Activity (A/20min./mg N) in Control Dogs				
Apex N=7	.921±.359	Base N=7	.814±.193	1.84 (paired)	N.S. >.05
	Regional Enzyme Activity (A/20min./mg N) in Shock Dogs				
Apex N=5	.393±.072	Base N=5	.358±.105	0.65 (paired)	N.S. >.05

TABLE 3

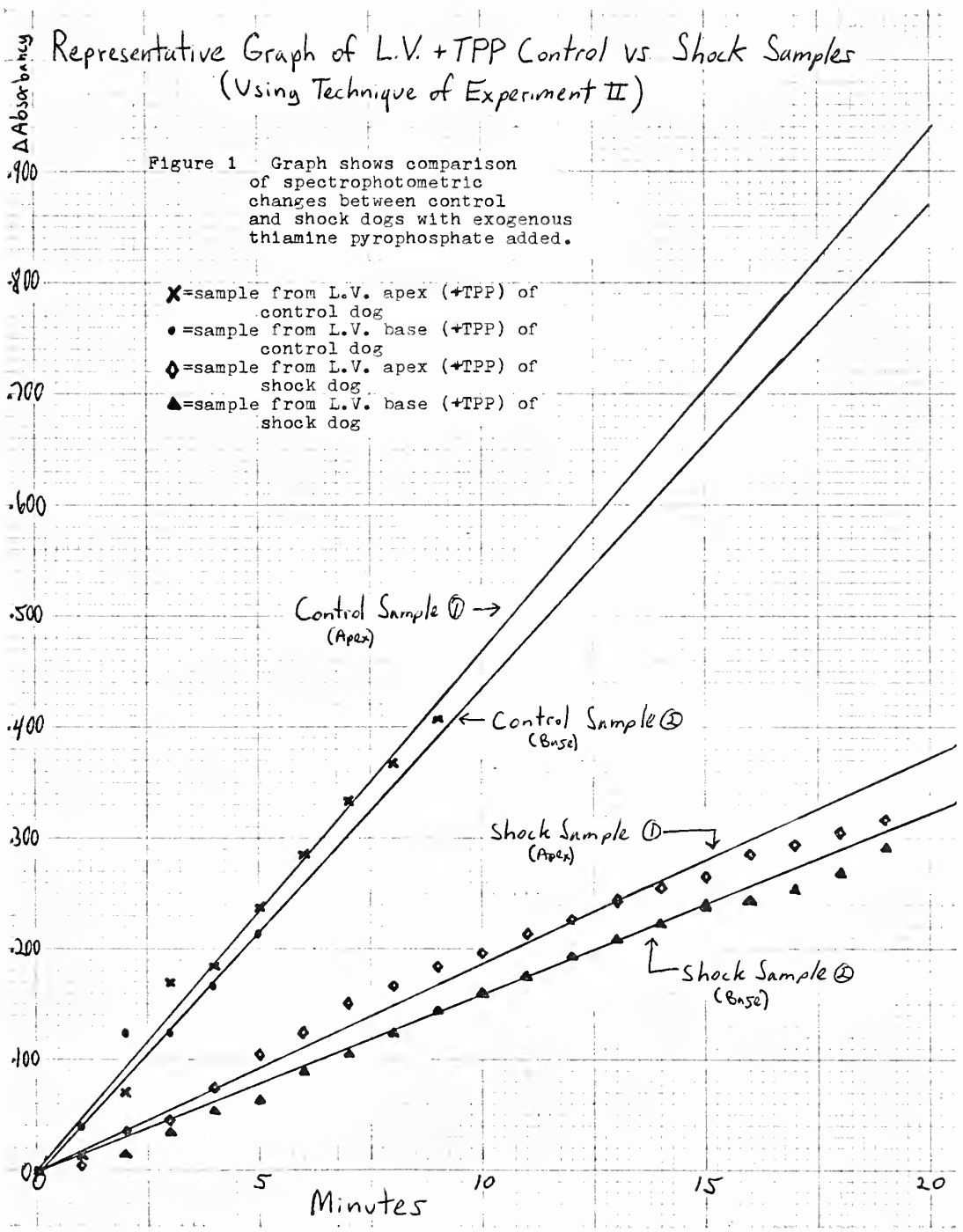
Results of Experiment III

Subject	Mean±SD	Subject	Mean±SD	t test	Probability
		A-V Control Dog Pyruvate (um/ml)			
Before Shock N=10	-0.038±.140	After Shock N=10	-0.038±.073	0.00 (paired)	N.S.* >.05
		A-V Shock Dog Pyruvate (um/ml)			
Before Shock N=7	-0.034±.061	After Shock N=7	-0.164±.087	3.23 (paired)	<.02
		A-V Control Dog Lactate (mg%)			
Before Shock N=10	12.05±5.74	After Shock N=10	11.66±4.81	0.20 (paired)	N.S. >.05
		A-V Shock Dog Lactate (mg%)			
Before Shock N=6	9.85±12.81	After Shock N=6	14.90±8.82	0.77 (paired)	N.S. >.05
		Pyruvate Dehydrogenase Complex Enzyme Activity (A/5min./mg N) With Thiamine Pyrophosphate			
Control Dogs N=10	.431±.096	Shock Dogs N=7	.321±.086	2.29	<.05
		Pyruvate Dehydrogenase Complex Enzyme Activity (A/5min./mg N) Without Thiamine Pyrosphosphate			
Control Dogs N=5	.453±.144	Shock Dogs N=5	.273±.080	2.16	N.S. >.05

*N.S. = not significant

TABLE 3 (Cont'd.)

Subject	Mean±SD	Subject	Mean±SD	t test	Probability
		Control Dogs' Enzyme Activity (A/5min./mg N)			
With Thiamine Pyrophosphate N=5	.423±.078	Without Thiamine Pyrophosphate N=5	.453±.144	0.50 (paired)	N.S. >.05
		Shock Dogs' Enzyme Activity (A/5min./mg N)			
With Thiamine Pyrophosphate N=5	.292±.086	Without Thiamine Pyrophosphate N=5	.273±.080	0.99 (paired)	N.S. >.05
		Regional Enzyme Activity (A/5min./mg N) in Control Dogs			
Apex N=5	.397±.095	Base N=5	.438±.111	1.41 (paired)	N.S. >.05
		Regional Enzyme Activity (A/5min./mg N) in Shock Dogs			
Apex N=2	.363±.128	Base N=2	.393±.008	0.23 (paired)	N.S. >.05


Representative Graph of L.V. +TPP Control vs Shock Samples
(Using Technique of Experiment II)
Figure 1 Graph shows comparison of spectrophotometric changes between control and shock dogs with exogenous thiamine pyrophosphate added.
✕=sample from L.V. apex (+TPP) of control dog
•=sample from L.V. base (+TPP) of control dog
◇=sample from L.V. apex (+TPP) of shock dog
▲=sample from L.V. base (+TPP) of shock dog
Δ Absorbancy
.900
.800
.700
.600
.500
.400
.300
.200
.100
0
Control Sample ① → (Apex)
← Control Sample ② (Base)
Shock Sample ① (Apex)
Shock Sample ② (Base)
0
5
10
15
20
Minutes

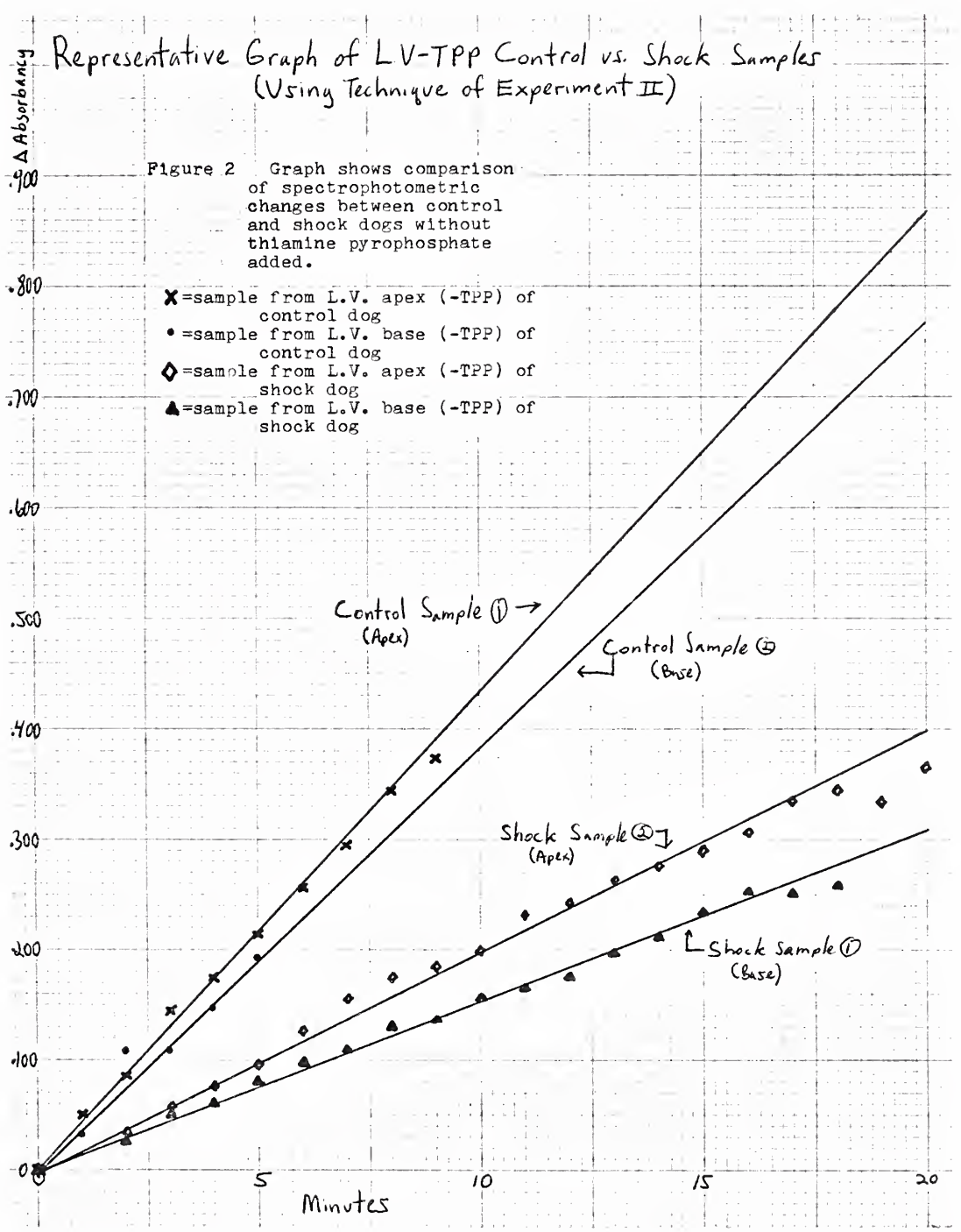


Figure 2 Graph shows comparison of spectrophotometric changes between control and shock dogs without thiamine pyrophosphate added.

✕ =sample from L.V. apex (-TPP) of control dog
• =sample from L.V. base (-TPP) of control dog
◇ =sample from L.V. apex (-TPP) of shock dog
▲ =sample from L.V. base (-TPP) of shock dog

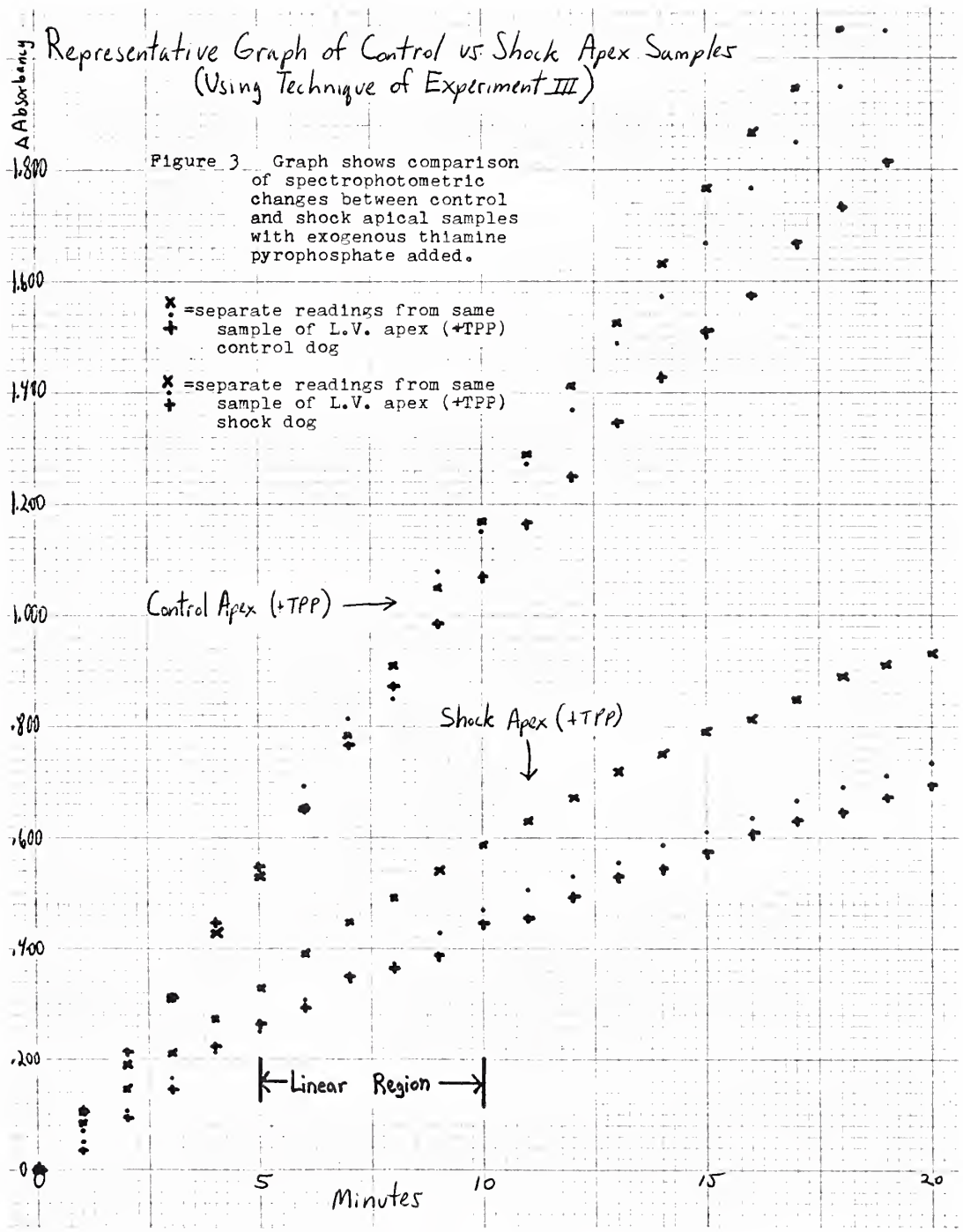


Figure 3 Graph shows comparison of spectrophotometric changes between control and shock apical samples with exogenous thiamine pyrophosphate added.

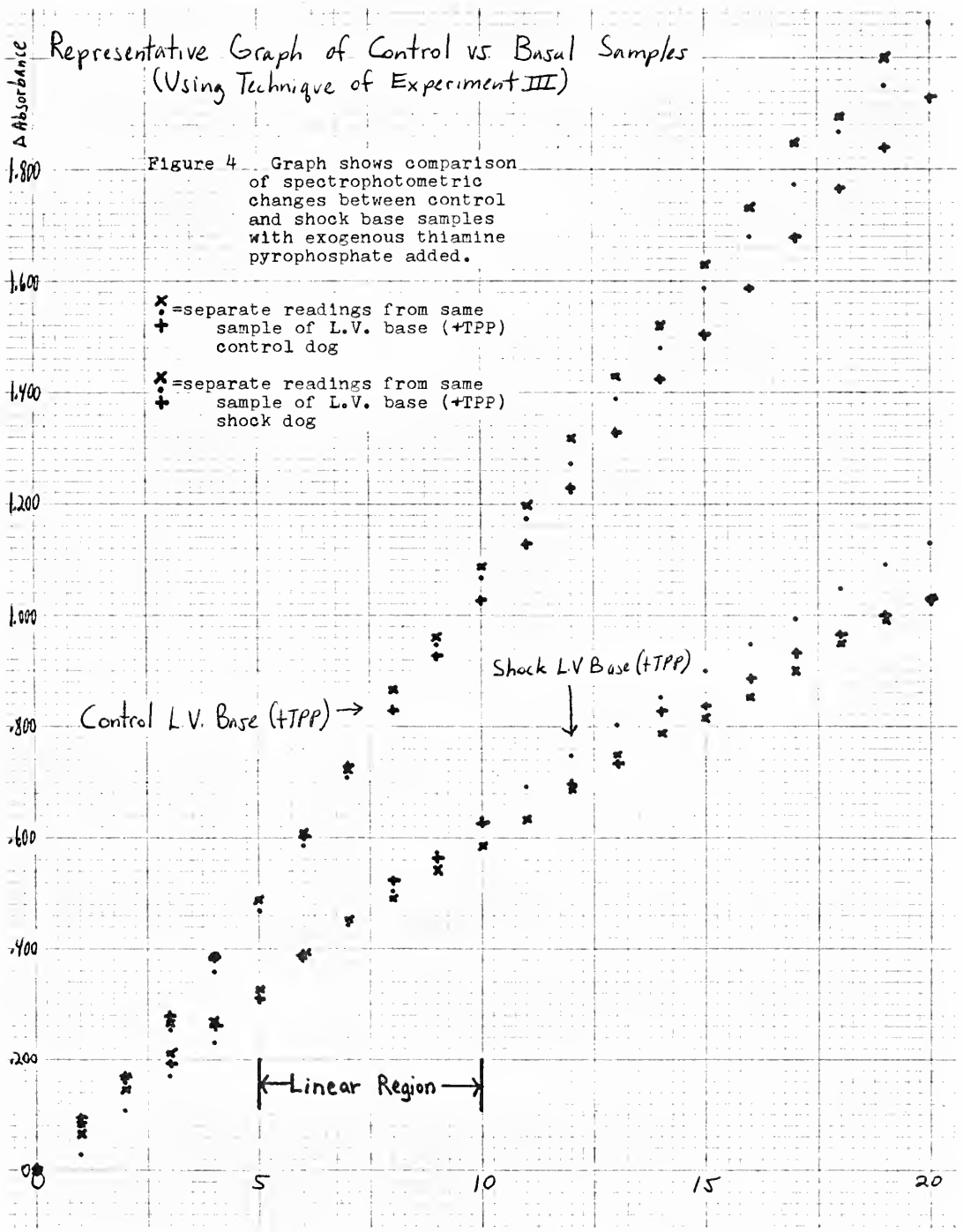


Figure 4. Graph shows comparison of spectrophotometric changes between control and shock base samples with exogenous thiamine pyrophosphate added.

DISCUSSION

The most striking metabolic abnormality observed in these experiments is the reversal of pyruvate extraction during early hemorrhagic shock. This finding is in agreement with the results of many other researchers.[46,50,51,52,53,54,55] Some control dogs were found to have an initial negative pyruvate extraction. In all of these dogs the pyruvate A-V difference became statistically more negative during shock. Normal dogs with negative pyruvate extractions have been reported elsewhere.[41,47,64] It has been observed that normal fasting dogs with low arterial pyruvate concentrations may have negative A-V pyruvate differences.[64] The present studies indicate that regardless of the initial myocardial pyruvate extraction, hemorrhagic shock persistently results in an increased pyruvate discharge by the myocardium.

Several researchers[50] have examined the problem of abnormal pyruvate metabolism in hemorrhagic shock and other conditions. They have focused much of their attention on thiamine metabolism and have concluded that a defect in cocarboxylase (thiamine pyrophosphate) is responsible for the observed abnormalities.

Govier and Greer[65] explored the effect of thiamine administration on survival time in hemorrhagic shock. By bleeding ten untreated and ten treated dogs to a mean

arterial blood pressure of 45-60mm Hg, they found that the thiamine treated dogs survived 2.4 times longer than the untreated group. In 70% of the treated group, a rise in blood pressure from five to 50mm Hg was seen 30 minutes after thiamine administration, lasting for one hour. Since thiamine is essential in pyruvate metabolism, Govier and Greer[66] also examined the blood levels of keto acids (pyruvate, alpha-ketoglutarate and oxaloacetate) before and after thiamine administration. They found increased levels of keto acids in the serum of shocked dogs which subsequently fell after the administration of thiamine, indicating better utilization of these substances. Govier[67] further examined the correlation between plasma thiamine levels and resistance to shock in dogs. He found: a) greater resistance to shock in dogs with high thiamine levels prior to shocking, b) that dogs containing higher thiamine levels withstood more bleeding before developing severe hypotension, c) that dogs fortified with thiamine showed a consistent tendency to return to normal blood pressure after hemorrhage while other dogs remained hypotensive, and d) that the incidence of intestinal hemorrhage after bleeding is much greater in dogs with low thiamine content. Finally, Govier and Greig[68] analyzed the biochemistry of thiamine utilization and noted that the phosphorylated form of thiamine (cocarboxylase) which acts as the coenzyme of pyruvate dehydrogenase is sensitive to hypoxia and can be destroyed by phosphotases in

the body. The demand for thiamine during hemorrhagic shock, they concluded, must be to counteract this phenomenon. Studying 11 control dogs and 19 shock dogs, they found a decrease in cocarboxylase in muscle, liver and duodenum during anoxia and hemorrhagic shock. Cocarboxylase (which diffuses out of cells once it is de-phosphorylated) could be re-synthesized in 30 to 60 minutes following the administration of thiamine. A deficiency of cocarboxylase would prevent carbohydrates from entering the TCA cycle via the conversion of pyruvate to acetyl-CoA.

Chiba et al.[69] looked at myocardial metabolism in transplanted homograft dog hearts. Glucose extraction was positive but lactate extraction was occasionally negative and pyruvate extraction was always negative, as in hemorrhagic shock. The respiratory quotient increased from 0.62 to 1.90 indicating a conversion of carbohydrates to fats. The redox potential was positive indicating the absence of anoxia. Thiamine hydrochloride was found to elevate the respiratory quotient and diminish the release of pyruvate from the myocardium. The authors hypothesize a block at the level of cocarboxylase following transplantation and that this block can be at least partially corrected with thiamine administration. The damage to cocarboxylase was probably not due to hypoxia but rather due to other pathologic mechanisms occurring in rejection reactions (i.e.

antibody response). As rejection became accelerated, malic dehydrogenase and other enzymes were found to be lost from the myocardial cells due to impaired cellular permeability. Wendt et al.[70] noted both negative myocardial lactate and pyruvate extraction in various myocardopathies such as idiopathic and alcoholic but could give no reason for this occurrence.

By employing a pyruvate dehydrogenase assay in the present study, it appears that the abnormal myocardial pyruvate metabolism seen in hemorrhagic shock is not due to a defect in cocarboxylase, but rather a defect in the enzyme complex, pyruvate dehydrogenase, itself. In experiments II and III control left ventricular enzyme activity was found not to change over a one hour period on a respirator. Yet, shock dogs had a statistically lower enzyme activity after one hour of hemorrhagic shock. There was no statistical difference between apical and left ventricular basal samples taken from the same dog. The addition of thiamine pyrophosphate (cocarboxylase) to the assay did not correct the decreased activity found in shock samples. While a defect in both cocarboxylase and pyruvate dehydrogenase cannot be excluded, it is certain that the pyruvate dehydrogenase enzyme complex must be defective, and this alone could account for decreased pyruvate utilization with subsequent pyruvate discharge from the myocardium. Furthermore, thiamine administration has not been found to correct this abnormality.

It is interesting to speculate on the possible causes of the enzymatic damage. While decreased blood flow is the primary insult in hemorrhagic shock, hypoxia, acidosis, increased catecholamines and possibly peripheral toxins follow closely. Very little research has been devoted to the influence of these factors on cardiac metabolism. However, certain correlations can be made between what is known of these factors in relation to survival, cardiac hemodynamics, cardiac metabolism, and the observations made in the present study.

A. Hypoxia

Crowell and Smith[71] have related oxygen deficit (difference between oxygen usage before and after shock) to survival. At an oxygen deficity of one hundred milliliters per kilogram all dogs survived, at one hundred and twenty milliliters per kilogram fifty per cent survived and at one hundred and forty milliliters per kilogram no dogs survived. The authors conclude that the amount of time spent in hemorrhagic shock is not as important as the oxygen deficit and this explains why some animals seem to survive better than others.

During periods of severe hypoxia, there is a build up of NADH in the myocardium which inhibits pyruvate dehydrogenase and forces a shift of pyruvate to lactate instead of acetyl-CoA.[72] Weil and Afifi[73] have found that oxygen

debt correlated best with the logarithm of lactate concentration. Lactate concentration in turn correlated excellently with decreased survival. Studying 142 human patients, they noted that as the arterial lactate concentration increased from 2.1 to 8 millimoles per liter, survival decreased from 90 to ten per cent. Huckabee[74] has defined the term "excess lactate" to correlate myocardial lactate production with severe hypoxia. Excess lactate takes into account changes in lactate production due to pyruvate concentration changes. Griggs et al.[75] have further shown that there is an oxygen gradient within the heart allowing one section (probably epicardium) to maintain oxidative pathways while other sections (probably endocardium) must rely on anearobic pathways. They used labeled lactate during periods of reduced blood flow to show that even while the myocardium is converting some lactate to carbon dioxide (labeled carbon dioxide) endogenous lactate is being produced.

Lemley and Meneely[76] have observed enzyme damage in hearts exposed to anoxic conditions. They report a decrease in coenzyme I and cytochrome c as well as a reduced rate of oxygen uptake in these hearts. Extracts of normal hearts were found to be capable of restoring previously anoxic hearts to normal. Danforth[77] noted a decreased pyruvate extraction during anoxia but Scheuer and Brachfield[78] report decreased fatty acid uptake with increased carbohydrate extraction and utilization. Shea

et al.[79] found a sudden increase in left atrial pressure (associated with heart failure) during lactate production by the heart under hypoxic conditions.

It is noteworthy that the decreased activity of pyruvate dehydrogenase described in the present study occurred during early hemorrhagic shock, before the myocardial production of lactate. At the time of negative pyruvate extraction, the arteriovenous lactate difference was actually greater than normal. Although there may have been severe hypoxia present in other organs, accounting for the increased arterial lactate concentration during shock, the increase in myocardial oxygen extraction (as evidenced by a decreased coronary sinus oxygen saturation in the presence of a normal or only slightly reduced arterial oxygen saturation) is probably responsible for preventing severe myocardial hypoxia and anaerobic metabolism.

The possibility of a lesser degree of hypoxia cannot be excluded and therefore it may be instructive to look at the response of the heart to milder forms of ischemia. Hackel et al.[80,81,82] reduced the ambient oxygen content of unanesthetized dogs from 21 to ten and then to five per cent. At both ten and five per cent, coronary blood flow, heart rate and the oxygen extraction coefficient increased so that myocardial oxygen utilization was well maintained. At five per cent oxygen the pyruvate and lactate arteriovenous differences all decreased (despite increased arterial

concentration). However, they never became negative and the increased coronary flow enabled total myocardial utilization of these substances to remain normal. Only during complete anoxia was there a negative arteriovenous difference in lactate accompanied by a further reduction in pyruvate extraction (and in one case a negative pyruvate extraction). On the other hand, in hemorrhagic shock, it has already been mentioned that McKeever et al.[52,53] could not prevent negative pyruvate extraction or irreversible shock from occurring despite maintenance of the coronary artery circulation. Also Ratlif et al.[54] used oxygen at high pressures and still found no success in correcting the abnormal pyruvate metabolism.

B. Acidosis

Scheuer[83] claims that excessive lactate production, associated with hypoxia, lowers intracellular pH and may damage enzymes or release lysosomes. Schumer[84] explored the problem of lactic acidosis in hemorrhagic shock. He infused normal dogs with lactic acid to the same concentration as is found in irreversibly shocked dogs (7.99 millimoles per liter) and found that all the animals died (at a pH of 6.8±.04). Photomicrographs showed the same microcirculatory picture as is seen in shock animals with a 65% blood loss (increased sludging, pooling, decreased flow). Goodyer et al.[85] studied the effects of alkalosis and

acidosis on myocardial metabolism. Alkalosis tended to increase coronary artery flow, cardiac output, arterial lactate concentration and arterial pyruvate concentration. Coincident with this, there was an increased uptake of lactate and pyruvate, and an increased utilization of lactate as an energy source. Acidosis tended to reduce coronary flow, cardiac output and myocardial oxygen consumption and increase the arterial glucose concentration. Although the concentration of glucose rose significantly, the myocardial extraction of glucose did not change, representing an inability to extract high levels of glucose during acidosis. Pyruvate and lactate extraction decreased. However, the proportion of energy yielded by carbohydrates was still high during acidosis and cardiac efficiency was not appreciably altered.

Baue et al.[86] while agreeing that acidosis in the isolated heart decreases myocardial contractility, reduces heart rate and decreases cardiac responsiveness to epinephrine and norepinephrine, showed that there were no significant differences in hemodynamic responses or oxygen consumption in shocked dogs treated with either sodium bicarbonate or sodium chloride, although the former returned the pH to normal (7.16 to 7.38). Nelson et al.[87] similarly showed by using the experimental drug tris (hydroxy-methyl) aminomethane, a powerful alkalinizing and diuretic agent,

that correction of pH alone does not increase survival in hemorrhagic shock.

During the period of increased negative pyruvate extraction and decreased pyruvate dehydrogenase activity described in this study, increased systemic lactate concentration and decreased pH were observed. Yetadministration of dextran corrected the negative pyruvate metabolism (at least temporarily) without changing the pH while administration of thiamine with sodium bicarbonate did not. Though this does not conclusively rule out the role of acidosis in damaging myocardial pyruvate metabolism, it does support the findings of Baue's and Nelson's groups. Since the pyruvate dehydrogenase enzyme was assayed under optimal in vitro conditions, including a potassium phosphate buffer, any enzyme damage due to acidosis would have been long lasting.

C. Catecholamines

Neither a review of the literature nor the experiments in this study can conclusively ascertain whether increased catecholamines in the circulation can cause myocardial damage on a biochemical level. The role of hyper-contractility and tachycardia in producing anatomical lesions of the myocardium has already been discussed.[29,30,31] The in vitro pyruvate dehydrogenase assay may be helpful in resolving this

problem by assaying left ventricular samples from dogs with and without heart block and/or beta blockade during hemorrhagic shock.

Goodale and Hackel[88] examined myocardial metabolism during periods of severe stress, when circulating adrenalin levels were very high, and found that lactate extraction to a major degree and pyruvate extraction to a minor degree were decreased. Hackel et al.[89] explored the effect of hypotension due to spinal anesthesia on myocardial metabolism. They noted that in a situation where reduced blood pressure led to a diminished coronary flow but no adrenalin release, glucose, lactate and pyruvate extractions all remained normal.

Simeone et al.[90] used a platinum electrode to measure oxygen tension in myocardial tissue during hemorrhagic shock with and without l-norepinephrine infusions. They found that if l-norepinephrine is given during the first five minutes of shock (before permanent damage due to hypoxia), not only is per cent survival increased, but oxygen tension in the myocardium is increased to 13% greater than normal during the infusion and slightly less than normal afterwards. This is opposed to a 61% decrease without the use of l-norepinephrine. The authors emphasize that they feel l-norepinephrine is helpful in early hemorrhagic shock but may be ineffective or even injurious if given

later in the course of hemorrhagic shock. Caliva et al.[91] used electropolarography to continuously record the availability of oxygen between the capillaries and muscle cells. They found that the oxygen availability fell during hemorrhagic shock to zero at a blood pressure of twenty millimeters Hg and that in early hemorrhagic shock any means of increasing flow (norepinephrine, dextran, plasma or blood) led to a restoration of normal oxygen availability. Thus any damage done to the myocardium on the basis of hypoxia could hopefully be prevented. Catchpole[92] found treatment with norepinephrine after 45 minutes of shock increased blood pressure, coronary blood flow and oxygen utilization without further increasing peripheral vasoconstriction. However the mortality remained the same with or without norepinephrine because of fatal cardiac arrhythmias induced by the norepinephrine infusion.

Entman et al.[93,94] found a greater systolic blood flow and decreased incidence of subendocardial necrosis when shock dogs were treated with pronethalol (a beta blocker). Baue et al.[95] contradict the significance of Entman's results by claiming increased survival (though not statistically significant) and increased cardiac output plus less mesenteric damage in dogs bled to thirty millimeters Hg and treated with isoproterenol (a beta stimulator).

D. Peripheral Toxins

The possibility of a peripheral toxin as discussed by Goodyer,[55] Lefer,[18] and Lillehei[8] also cannot be ruled out. Although several different end-organs and types of toxins have been hypothesized, all authors agree that peripheral ischemia is the initiating factor. Based on this assumption, one would expect harmful effects from systemic vasoconstrictors during shock and helpful effects with the use of vasodilators. Most of the experimental work on this subject unfortunately remains inconclusive.

Opdyke[96] bled dogs to a mean arterial blood pressure of fifty millimeters Hg for 90 minutes, then re-bled them to thirty millimeters Hg. After three minutes he infused neo-synephrine to increase the blood pressure to between fifty and seventy-five millimeters Hg for 45 minutes and then transfused all dogs with their own blood. The average length of survival of the treated dogs was 13.4 hours while that for the untreated dogs was only 5.3 hours. He concluded that any peripheral damage that might be done by intense vasoconstriction is more than compensated for by increased flow through vital centers, thereby increasing survival time. A contrary view is held by Hackel[97] who infused l-norepinephrine after 45 minutes of shock and noted no increased survival or cardiac output but did notice increased peripheral vascular resistance. All of these parameters could be corrected by infusing whole blood. He subscribes to the

theory that peripheral ischemia may cause the release of a toxin which can affect myocardial enzyme systems. Hackel[98] also observed an increased incidence of subendocardial hemorrhage in shock dogs treated with l-norepinephrine, probably due to the increased work load placed on the heart. Remington et al.[99] found a seven to nine times greater survival in dogs pre-treated with dibenamine (a sympathetic blocker-vasodilator). They hypothesize that dibenamine prevents low flow to a vital organ during shock which is necessary for survival. Weil and Whighan[100] discovered an increased survival in rats given 100 times the replacement dose of glucocorticoids and ascribed this to the vasodilatory effects of steroids. Danoff and Greene,[101] however, report increased excess lactate with hexamethonium (a ganglionic sympathetic blocker-vasodilator) and Glasser and Page[102] notes no increase in survival with the use of tetramethyl ammonia (a sympathetic blocker-vasodilator).

Further studies along these lines could be accomplished by examining dogs in whom the systemic circulation is maintained while the coronary circulation remains at shock levels and vice versa. The release of peripheral toxins would be expected to occur only during systemic ischemia. Assaying for pyruvate dehydrogenase activity under these circumstances may demonstrate what relationship, if any, exists between peripheral toxins and myocardial enzyme damage. Similarly, one could directly assay the effect of plasma from shocked

dogs on the pyruvate dehydrogenase activity of left ventricular samples from normal dogs.

Summary

The present study highlights one early metabolic derangement of the myocardium during hemorrhagic shock. The significance of this defect and its possible causes can only be presently guessed at. Since the decreased activity of the pyruvate dehydrogenase enzyme complex occurs during a period of aerobic myocardial metabolism (before lactate production) one would expect deficient or inefficient energy production. It is worthwhile noting that several researchers have described a decrease in myocardial performance during shock[24,25] and that the role of myocardial failure in irreversible shock remains to be settled. A reduction in the activity of pyruvate dehydrogenase may be one of the early signs of myocardial damage and understanding the causes of this reduced activity (acidosis, catecholamines, hypoxia or toxins) may eventually lead to a more precise understanding of the patho-physiology of hemorrhagic shock and to better modes of treatment.

CONCLUSION

The following observations have been made in this study:

1. Within one hour after hemorrhagic shock in dogs (b.p. 25-45mm Hg) the myocardial arteriovenous pyruvate difference either becomes negative or if negative to start with, becomes significantly more negative. At the same time, the myocardial arteriovenous lactate difference remains positive.

2. During hemorrhagic shock, myocardial oxygen extraction is increased, arterial lactate and pyruvate concentrations are increased, and arterial pH is decreased.

3. Infusions of thiamine hydrochloride or cocarboxylase during hemorrhagic shock does not correct the negative myocardial pyruvate extraction.

4. Infusions of 6% dextran in normal saline, which returns the mean arterial blood pressure to normal, corrects the negative myocardial pyruvate extraction at least temporarily.

5. Returning shed blood after three hours of hemorrhagic shock can sometimes restore positive myocardial pyruvate extraction.

6. A method of assaying the pyruvate dehydrogenase enzyme complex in myocardial tissue has been established.

This assay is based on the photometric change observed as ferric ions are reduced to ferrous ions and is expressed as delta A/5min./mg N.

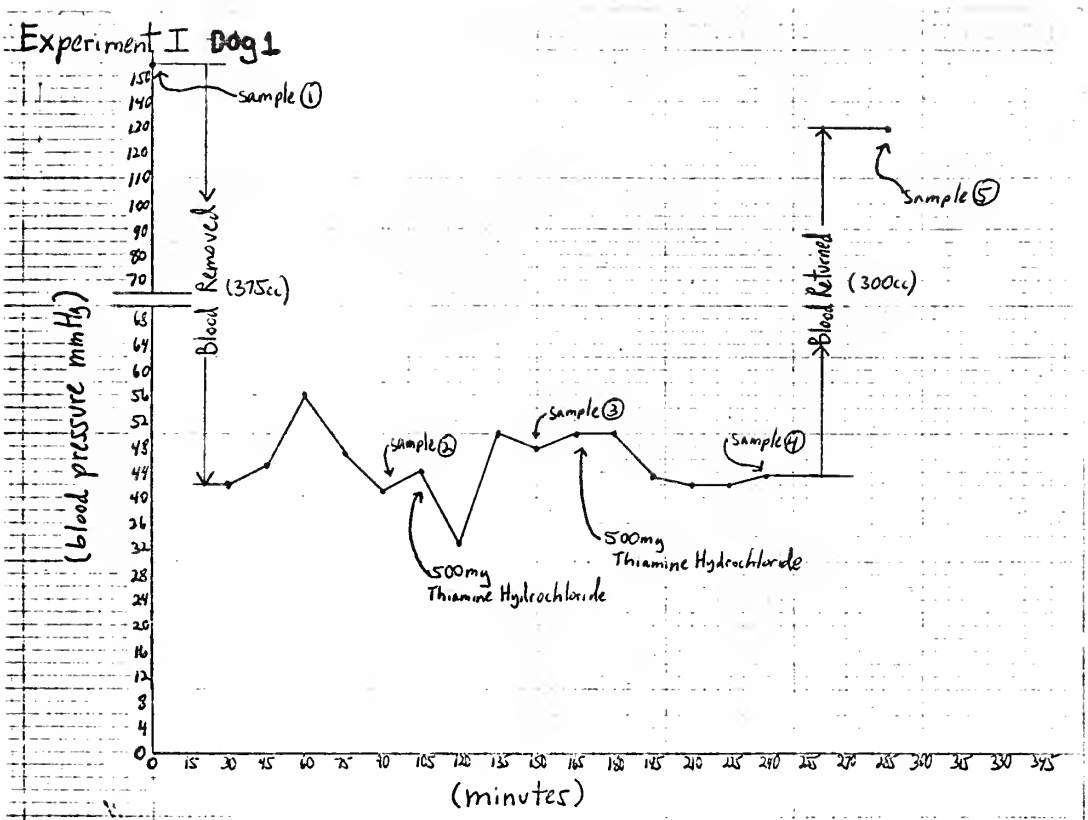
7. After one hour on a respirator, there is no change in left ventricular pyruvate dehydrogenase activity but after one hour of hemorrhagic shock, this activity is significantly decreased in both apical and basal left ventricular samples.

8. Exogenous cocarboxylase added to the assay does not affect enzyme activity.

9. Decreased pyruvate dehydrogenase activity may represent an early form of myocardial damage during hemorrhagic shock. Acidosis, hypoxia, catecholamines and peripheral toxins have been discussed as possible causes of this damage.

10. In the future, pyruvate dehydrogenase and other enzyme assays may be used to determine which agents are responsible for myocardial damage and perhaps irreversible hemorrhagic shock. Derangements in myocardial metabolism may precede contractility or hemodynamic impairments. Further study of myocardial metabolism is encouraged.

APPENDIX



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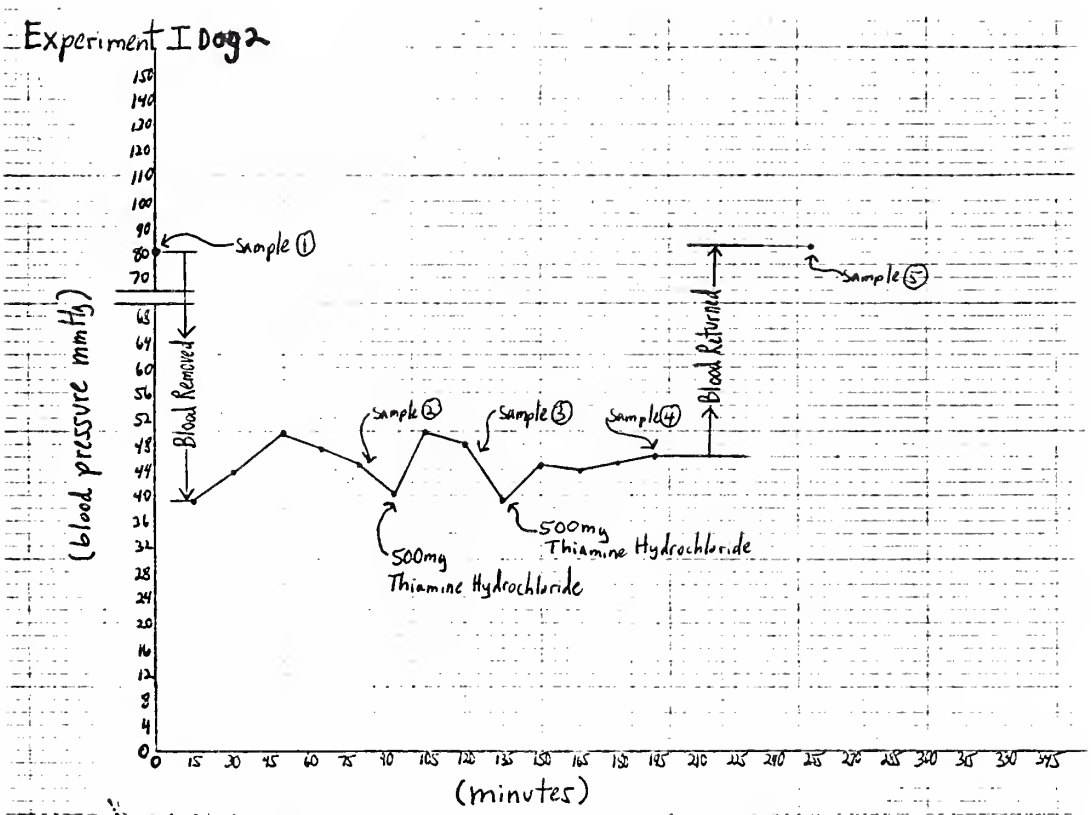
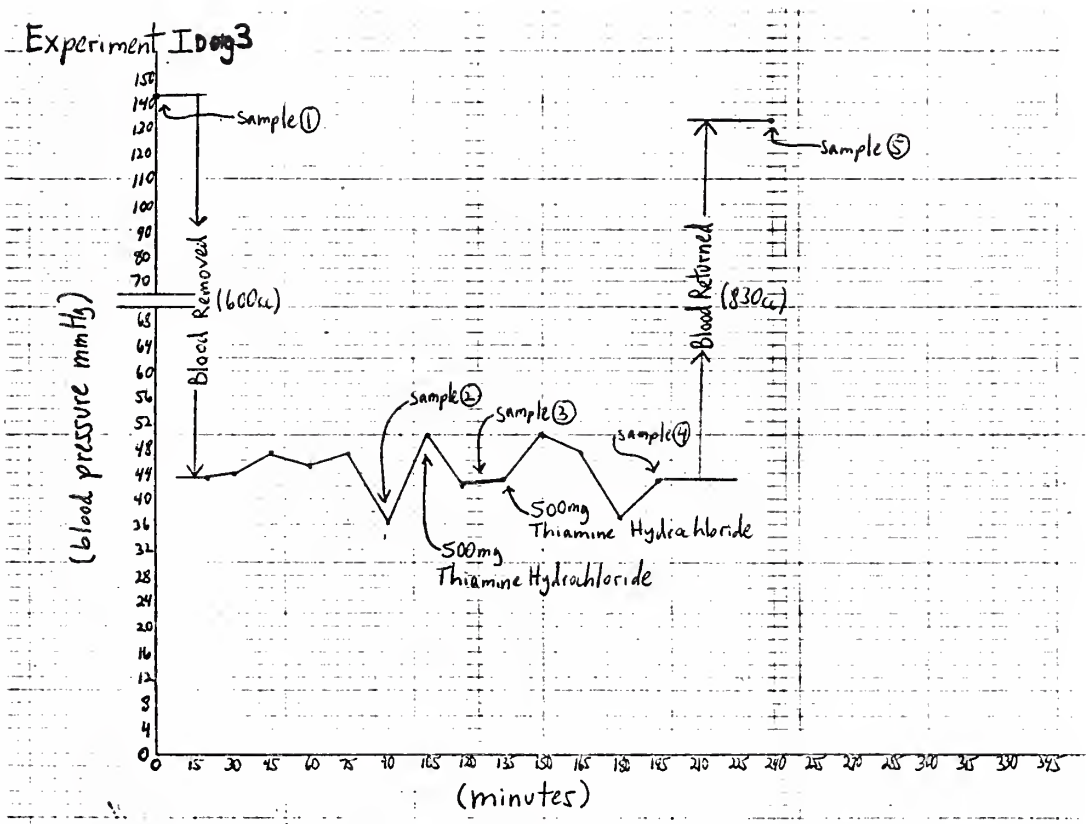
2	Art	50.889	.0967	97.0	14.0	
	CS	46.377	.1024	21.0	13.5	
3	Art	51.265	.1254	94.5	13.2	
	CS	36.519	.1648	35.0	13.2	
4	Art	43.228	.1424	94.5	15.6	
	CS	24.981	.1020	35.0	15.5	
5	Art	42.676	.1091	94.5	19.0	
	CS	—	.2331	47.0	18.2	
6	Art					
	CS					
7	Art					
	CS					



		LACTATE mg/100ml	PYRUVATE µm/ml	%O_2	Hgb. mg/100ml	pH
1	Art.	23.733	.1363	97.5	17.8	
	C.S	14.065	.0742	24.0	17.6	
2	Art.	24.287	.1306	96.5	16.5	
	C.S	14.194	.0992	29.5	16.3	
3	Art.	26.661	.1486	96.0	16.2	
	C.S	17.729	.1101	41.0	17.0	
4	Art.	23.324	.1812	97.2	15.8	
	C.S	12.526	.1288	32.5	16.2	
5	Art.	28.024	.0866	97.5	11.9	
	C.S	18.271	.1357	44.5	17.7	
6	Art					
	C.S					
7	Art					
	C.S					



		LACTATE mg/100ml	PYRUVATE mm/ml	% O_2	Hgb. mg/100ml	pH
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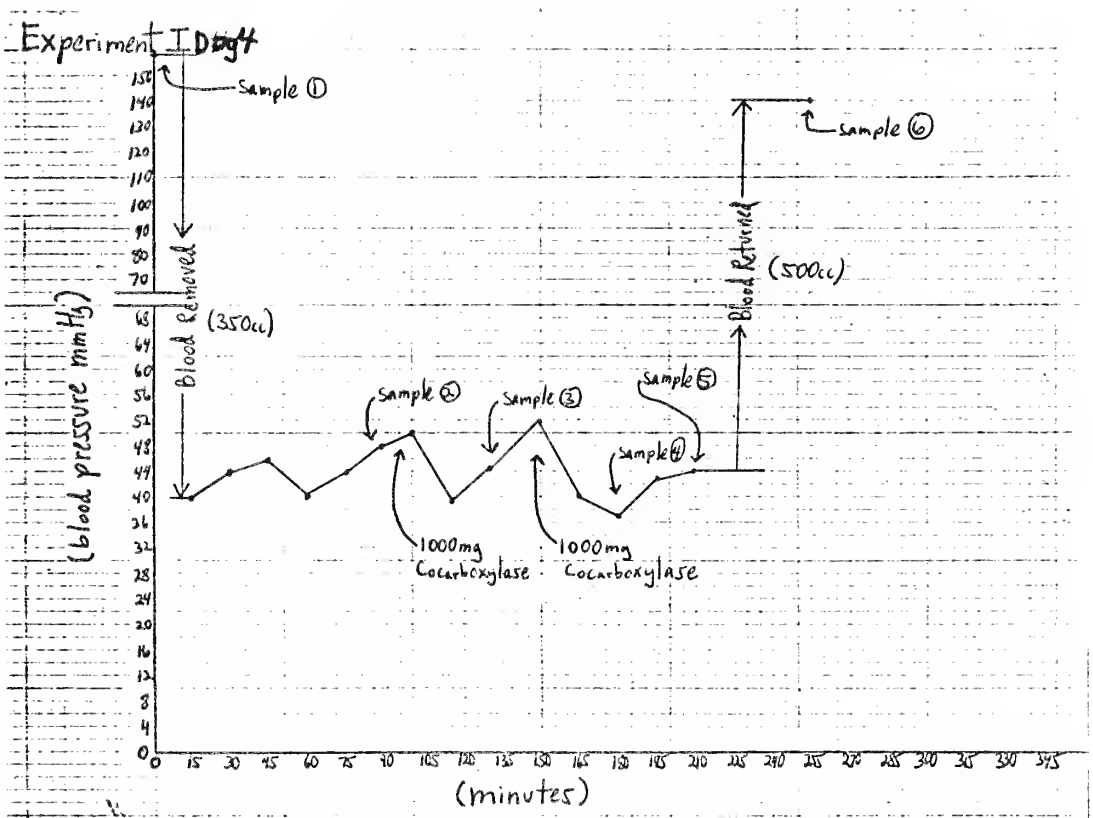
	C.S.	4.630	.0262	36.0	12.6	
2	Art.	57.763	—	94.2	11.2	
	C.S.	44.650	—	28.0	10.5	
3	Art	54.179	.0769	94.5	9.8	
	C.S.	42.829	.0984	27.0	10.2	
4	Art.	57.869	.0854	95.0	10.2	
	C.S.	43.863	.1689	26.0	10.1	
5	Art.	43.569	.1846	98.0	13.0	
	C.S.	28.564	.0709	36.5	13.5	
6	Art					
	C.S.					
7	Art.					
	C.S.					



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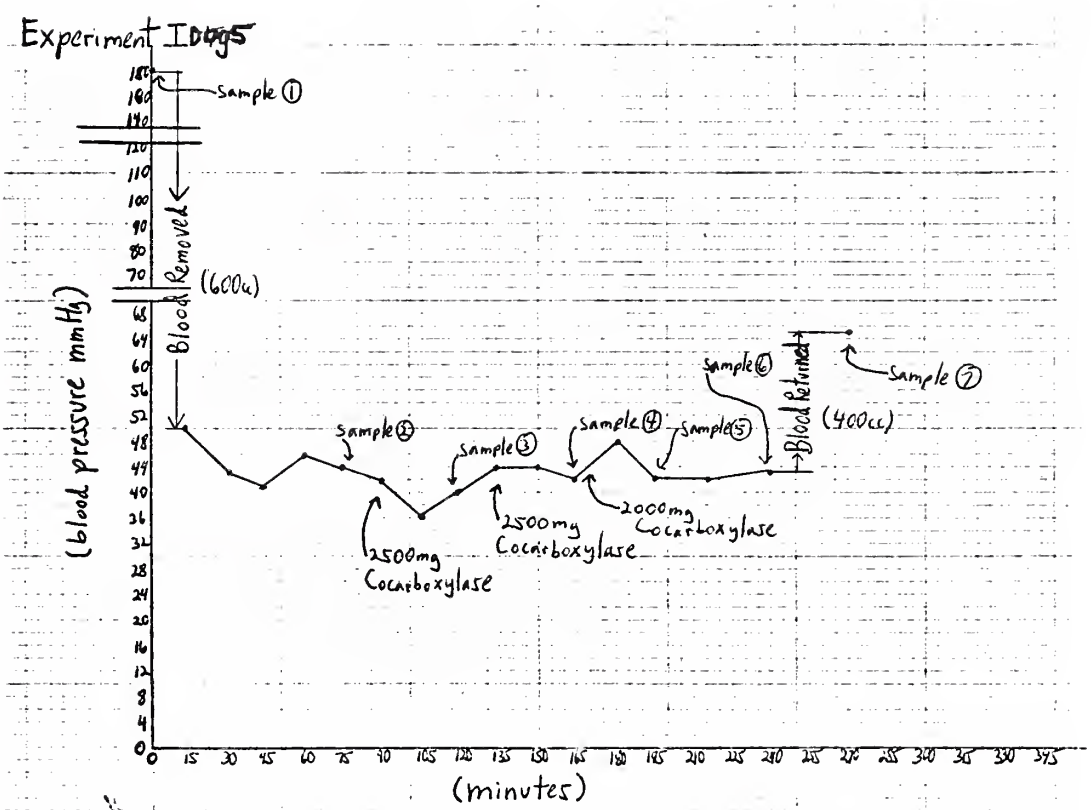
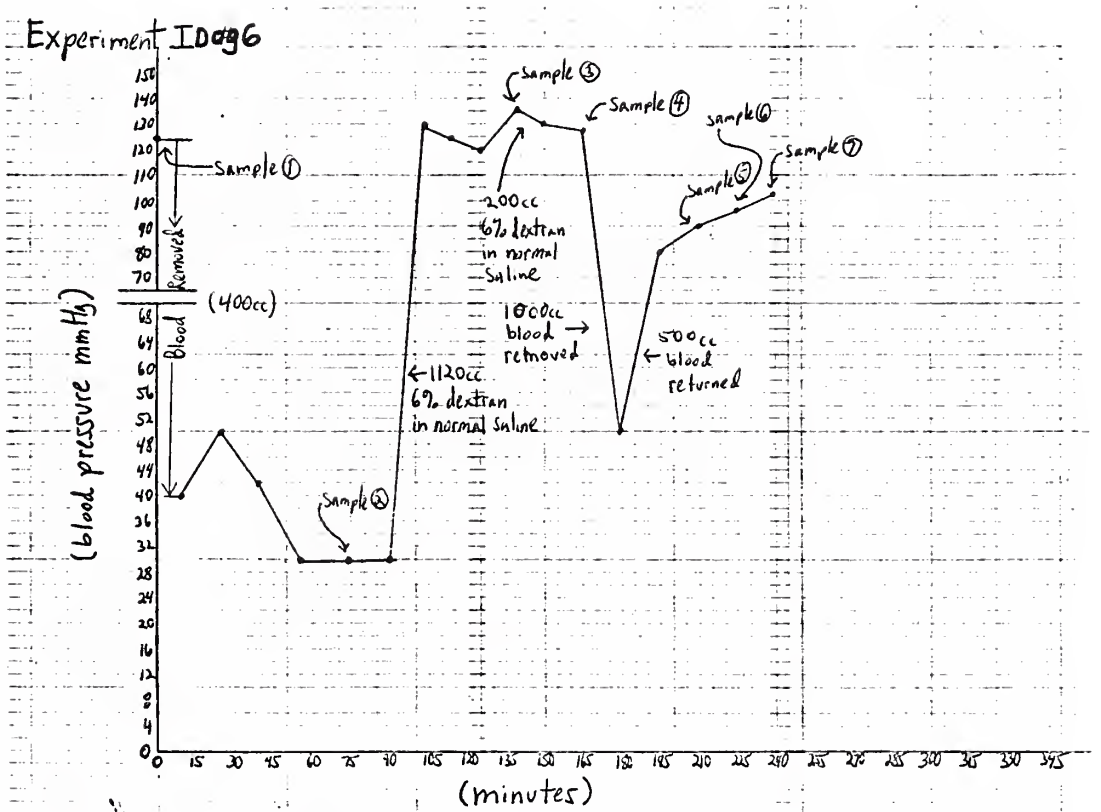
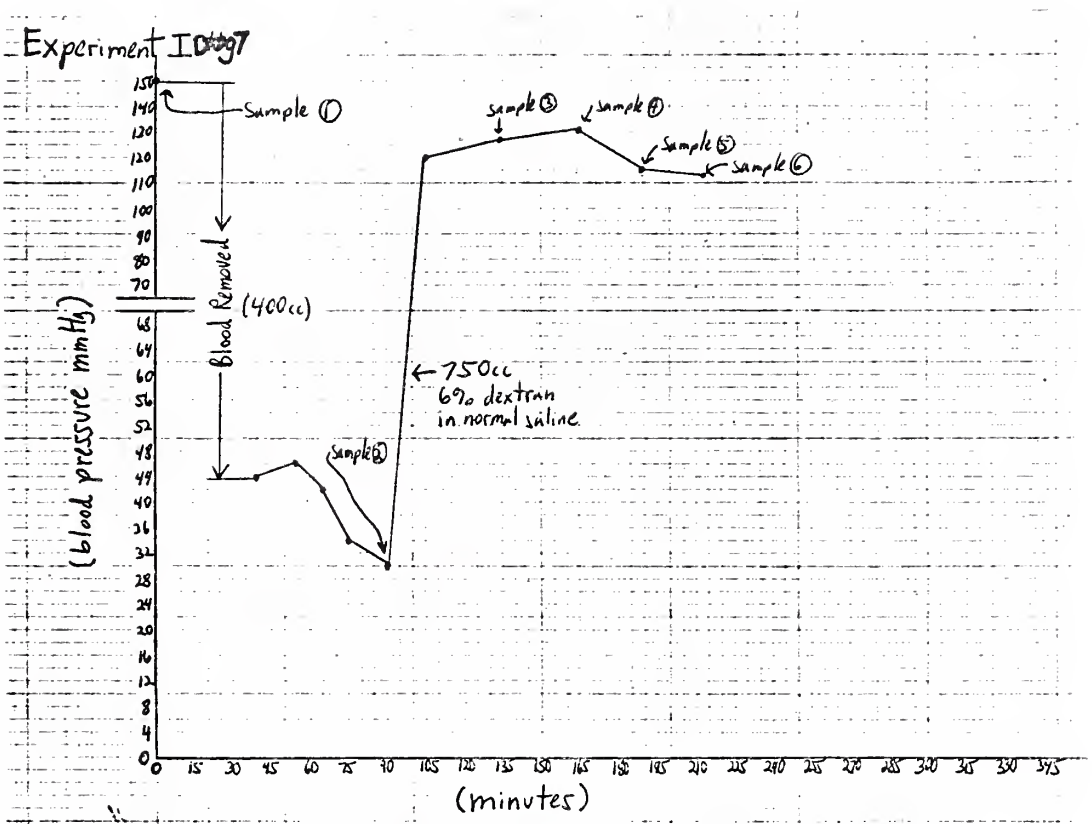
1	Art.	28.118	.2628	97.5	18.2	
	C.S	28.494	.2208	33.0	18.5	
2	Art.	102.143	.1797	94.0	12.1	
	C.S	93.001	.2755	25.0	11.9	
3	Art.	99.017	.1566	94.5	10.8	
	C.S	82.215	.2566	31.0	10.8	
4	Art.	71.887	.1746	94.8	10.9	
	C.S	60.407	.2752	32.0	11.0	
5	Art.	57.281	.0851	95.2	11.9	
	C.S	46.166	.1121	32.0	11.5	
6	Art	64.190	.2099	96.5	18.2	
	C.S	45.578	.2075	37.0	18.4	
7	Art					
	C.S					



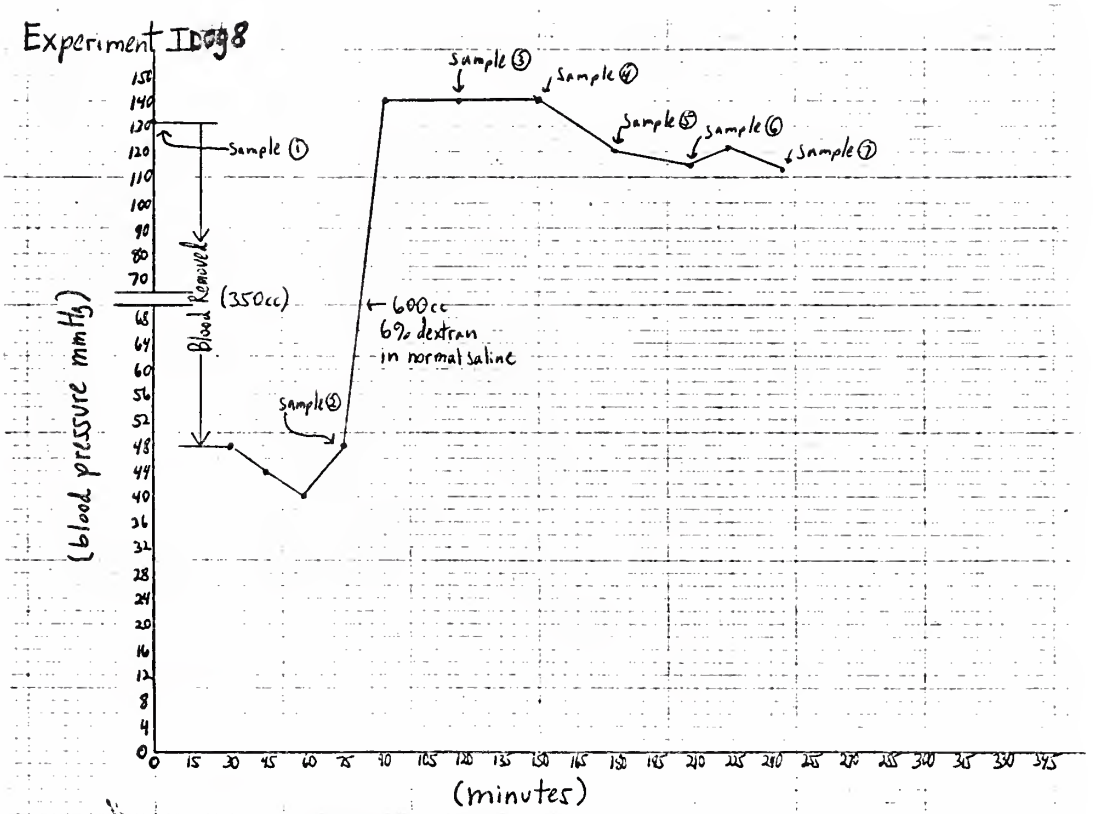
		LACTATE mg/100ml	PYRUVATE μm/ml	%O_2	Hgb. mg/100ml	pH
1	Art.	26.0263	.0881	98.5	16.4	
	C.S	15.0753	.0814	45.0	16.3	
2	Art.	110.0388	.0242	97.2	13.7	
	C.S	106.7135	.1599	20.0	13.5	
3	Art	111.3783	.0463	97.2	15.0	
	C.S	100.9413	.2085	24.0	15.0	
4	Art	108.6523	.1835	97.2	16.3	
	C.S	100.2158	.2290	31.0	16.0	
5	Art.	103.6350	.1462	97.5	16.0	
	C.S	100.0865	.1754	32.5	16.0	
6	Art	102.6010	.1451	98.5	17.0	
	C.S	92.7898	.1852	24.0	17.1	
7	Art	101.3085	.1085	98.5	16.7	
	C.S	93.8943	.1615	44.0	16.5	



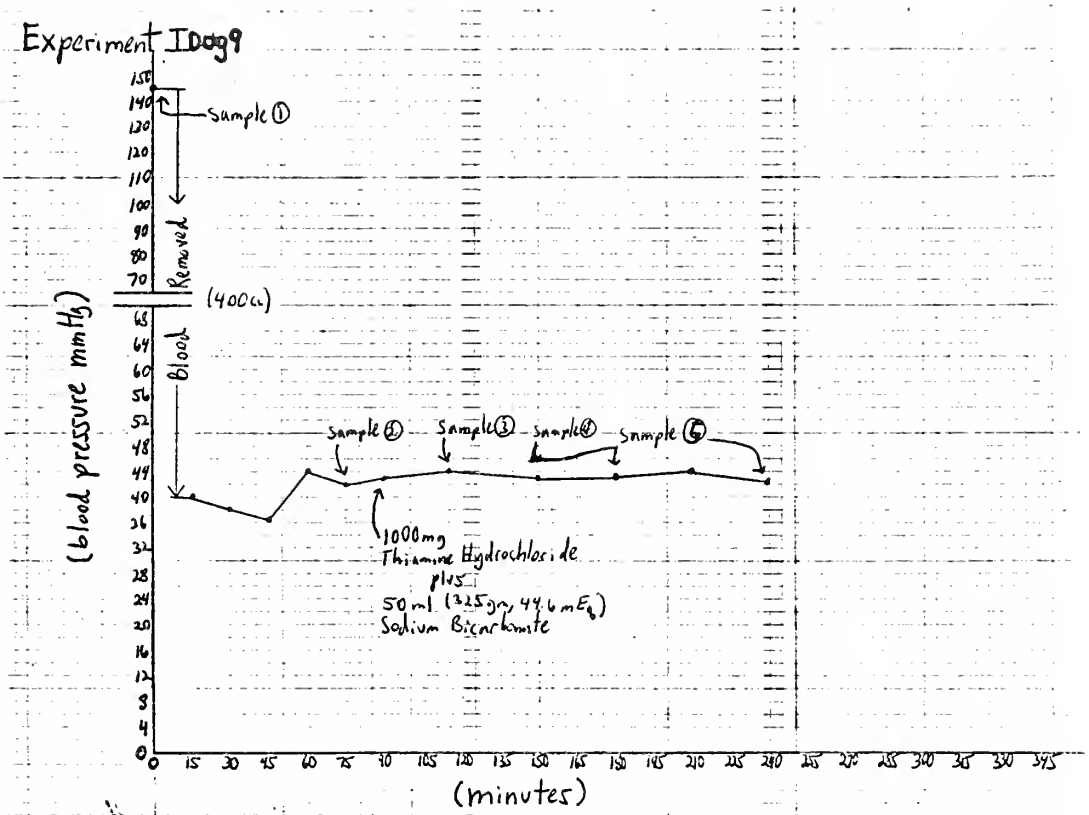
		LACTATE mg/100ml	PYRUVATE um/ml	% O_2	Hgb. mg/100ml	pH
1	Art.	45.414	.3624	98	15.0	
	C.S.	33.924	.1891	34	15.6	
2	Art.	89.077	.1323	97	13.6	
	C.S.	82.285	.2324	<20	13.6	
3	Art.	92.155	.2267	96	<4	
	C.S.	90.905	.2376	52.2	<4	
4	Art.	85.376	.2729	95.5	<4	7.305
	C.S.	85.525	.2673	55.0	<4	7.290
5	Art.	84.424	.1605	94.2	9.4	
	C.S.	77.139	.2212	41	9.3	
6	Art.	80.922	.2413	93.5	9.2	7.225
	C.S.	75.494	.2664	37	9.0	7.235
7	Art.	76.399	.1757	89.2	9.3	7.220
	C.S.	71.193	.1944	35.5	9.3	7.250



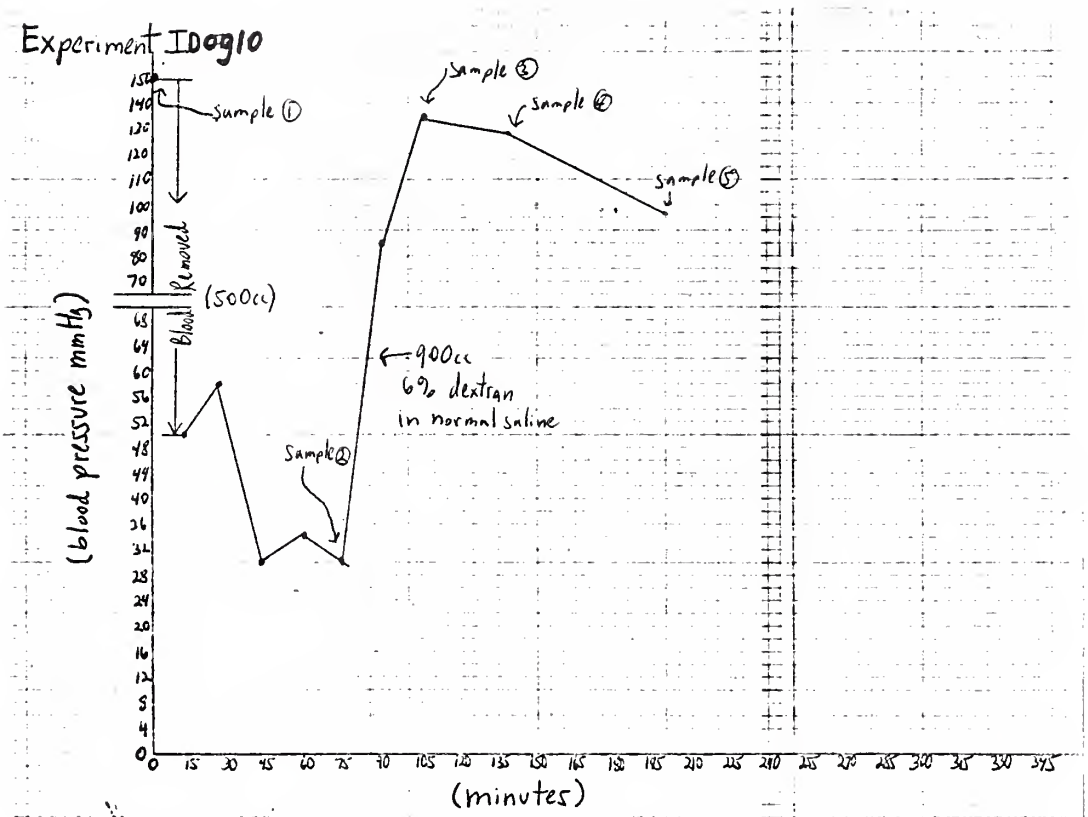
		LACTATE mg/100ml	PYRUVATE mm/ml	% O_2	Hgb. mg/100ml	pH
1	Art.	39.480	.4553	97.5	16.6	
	C.S	28.717	.1972	48.5	17.0	
2	Art.	88.102	.0951	97.0	12.6	
	C.S	90.522	.1514	20.0	12.5	
3	Art.	53.263	.6126	96.5	<4	7.430
	C.S	52.464	.5662	56.5	<4	7.385
4	Art.	52.652	.6056	95.8	<4	7.520
	C.S	53.416	.5994	51.5	<4	7.490
5	Art.	42.418	.4758	96.0	4.1	7.660
	C.S	43.898	.4424	48.5	4.4	7.595
6	Art.	29.845	.3600	96.5	4.6	
	C.S	31.878	.3486	46.5	4.7	
7	Art					
	C.S					



		LACTATE mg/100ml	PYRUVATE mm/ml	$\%O_2$	Hgb. mg/100ml	pH
1	Art	40.402	—	98.5	19.5	
	C.S	22.607	.1359	35.0	19.9	
2	Art	70.958	.2204	95.5	15.4	7.447
	C.S	58.515	.2171	28.0	15.8	7.375
3	Art	57.458	.1562	97.5	7.4	
	C.S	44.914	.1160	48.0	7.6	7.75
4	Art	45.473	.1790	98.0	8.1	7.72
	C.S	36.660	.1343	51.2	8.0	
5	Art	37.377	.1216	97.5	7.8	
	C.S	30.550	.1153	50.8	7.7	
6	Art	34.392	.1651	98.2	7.1	
	C.S	28.476	.1577	51.2	7.2	
7	Art	37.436	.2293	98.2	7.5	7.835
	C.S	30.609	.2043	46.5	8.0	7.817



		LACTATE mg/100ml	PYRUVATE um/ml	%O_2	Hgb. mg/100ml	pH
1	Art	18.612	.1436	97.5	13.6	
	C.S	10.105	.1026	27.5	13.6	
2	Art	72.615	.0924	97.5	12.9	7.300
	C.S	61.053	.1754	<20	12.7	7.210
3	Art	88.642	.2128	97.2	12.0	
	C.S	77.303	.2759	24.0	12.0	7.440
4	Art	79.618	.2789	97	14.3	7.345
	C.S	65.412	.2777	<20	14.3	
5	Art	78.561	.2875	98	16.2	
	C.S	63.297	.3317	21	16.4	7.415
6	Art					
	C.S					
7	Art					
	C.S					



		LACTATE mg/100ml	PYRUVATE um/ml	$\%O_2$	Hgb. mg/100ml	pH
1	Art	38.470	.1545	97	16.7	
	C5	20.375	.0836	26.5	16.7	
2	Art	82.732	.1161	97.2	13.2	7.170
	C5	72.439	.1601	20.0	13.8	7.135
3	Art	90.757	.1896	96.0	9.1	
	C5	102.777	.1868	40.0	5.4	
4	Art	77.747	.1852	95.8	4.4	7.285
	C5	69.959	.1536	43.0	5.1	7.285
5	Art	46.847	.2715	94.8	5.4	
	C5	42.406	.2418	38.0	5.6	
6	Art					
	C5					
7	Art					
	C5					

Experiment II

Left Ventricular Enzyme Activity
(A/20min./mg N)

Dog	Apex		Base*	
	+TPP	-TPP	+TPP	-TPP
	Before Shock			
1C	.601	.520	.719	.708
2C	.590	.590	.671	.731
3C	.869	.862	.769	.660
4C	1.604	1.590	1.224	1.230
5C	.922	.868	.871	.794
6C	1.222	1.344	.980	.800
7C	.662	.662	.610	.630
	After Shock			
1S	.303	.316	.408	.399
2S	.382	.481	.540	.462
3S**	.259	.224	.536	.505
4S	.495	.500	.248	.215
5S***	.749	.740	.1210	.1020
6S	.329	.329	.462	.308
7S	.454	.438	.279	.250

*After one hour on a respirator
**Dog died during experiment
***Negative lactate extraction during shock

Experiment II

Pyruvates (um/ml)

Dog	Before Shock Art.	C.S.	A-V Diff.	After Shock Art.	C.S.	A-V Diff.
1C						
2C						
3C						
4C	.0155	.0608	-.0453			
5C	.0586	.0877	-.0291			
6C	.0421	.0314	-.0107			
7C	.0325	.0796	-.0471			
1S	.0958	.1815	-.0857	.0576	.1962	-.1386
2S	.2069	.2814	-.0745	.1322	.1895	-.0573
3S	.0863	.1396	-.0533		Dog Died	
4S	.0168	.1065	-.0897	.0475	.1637	-.1162
5S	.0822	.1876	-.1054	.0287	.1507	-.1220
6S	.1170	.0860	0.031	.0420	.2020	-0.160
7S	.0029	.0649	-.0612	.0908	.3954	-.2046

Experiment II

Lactates (mg/100ml)

	Before Shock			After Shock		
Dog	Art.	C.S.	A-V Diff.	Art.	C.S.	A-V Diff.
1C						
2C						
3C						
4C	11.136	6.518	4.618			
5C	31.883	20.114	11.769			
6C	8.069	4.077	3.992			
7C	15.737	8.728	7.009			
1S	13.970	14.984	-1.014	54.169	39.320	14.849
2S	38.599	30.027	8.572	55.756	38.498	17.258
3S	20.230	15.600	4.630	Dog Died		
4S	9.197	4.687	4.510	29.697	22.537	7.160
5S	47.383	46.981	0.402	75.891	90.624	-14.733
6S	36.590	18.150	18.440	**	88.630	
7S	8.602	7.216	1.386	86.521	79.499	7.022

**sample lost

Experiment II

Dog	% O_2 Sat. Before Shock Art.	C.S.	After Shock Art.	C.S.	B.P. (mmHg) Before Art.	After Art.	Approx. Blood Loss (cc)
1C							
2C							
3C							
4C	94	28			116.5		
5C	98	39			174		
6C	92	30			139		
7C	96	25			121.5		
1S	100	52	98	30	117	43-47	600
2S	100	49	100	20	97	25-44	550
3S	99	50	Dog Died		125	30-47	620
4S	99	30	94	<20	95	31-38	650
5S	96	41	93	<20	71	23-45	560
6S	95	32	93	<20	75	24-31	650
7S	96.5	26.5	45	<20	145	37-47	550

Experiment III

Left Ventricular Enzyme Activity
(A/5min./mg N)

Dog	Control A	Control B	Control C	Dog	Shock A	Shock B	Shock C	Avg.* Control	Avg.* Shock
1C				1S					
apex+TPP	.320	.370		apex+TPP	.260	.420		.345	.340
base+TPP	.270	.320		base+TPP	.380	.240		.295	.310
2C				2S					
apex+TPP	.250	.330	.400	apex+TPP	.270	.280		.365	.275
base+TPP	.380	.410		base+TPP	.400	.420		.395	.410
3C				3S					
apex+TPP	.420	.460	.440	apex+TPP	.370	.500	.480	.450	.490
base+TPP	.500	.590	.550	base+TPP	.380	.410	.390	.570	.385
4C				4S					
apex+TPP	.570	.630	.530	apex+TPP	.230	.240	.180	.550	.235
base+TPP	.570	.590	.560	base+TPP	.400	.400	.400	.565	.400
5C				5S					
apex+TPP	.280	.220	.270	apex+TPP	.290	.260	.280	.275	.285
base+TPP	.330	.360	.370	base+TPP	.320	.260	.290	.365	.305
6C				6S					
base-TPP	.550	.590	.670	base-TPP	.350	.300	.380	.570	.365
base+TPP	.550	.510	.500	base+TPP	.340	.360	.430	.530	.350

*Avg. = The arithmetic average of two closest values

Experiment III

Left Ventricular Enzyme Activity (cont'd.)

Dog	Control A	Control B	Control C	Dog	Shock A	Shock B	Shock C	Avg. Control	Avg. Shock
7C				7S					
base-TPP	.150	.290	.290	base-TPP	.320	.350	.370	.290	.360
base+TPP	.290	.230	.290	base+TPP	.410	.420	.490	.290	.415
8C				8S					
base-TPP	.610	.610	.460	base-TPP	.280	.200	.250	.610	.265
base+TPP	.550	.420	.400	base+TPP	.250	.300	.300	.410	.300
9C				9S					
base-TPP	.300	.550	.500	base-TPP	.160	.180	.160	.525	.160
base+TPP	.440	.510	.440	base+TPP	.150	.220	.210	.440	.215
10C				10S					
base-TPP	.270	.270	.270	base-TPP	.270	.270	.165	.270	.217
base+TPP	.470	.420	.310	base+TPP	.390	.200	.170	.445	.180

Experiment III

Pyruvates (um/ml)
(control dogs)

Dog	Before 1 Hour Art.	C.S.	A-V Diff.	After 1 Hour Art.	C.S.	A-V Diff.
1C	0.0568	0.0611	-0.00534	0.0951	0.1083	-0.01318
2C	0.0557	0.0405	+0.0152	0.1130	0.1570	-0.0440
3C	0.0267	0.1751	-0.1484	0.1855	0.2934	-0.1079
4C	0.1026	0.1431	-0.405	0.2241	0.1009	0.1232
5C	0.0277	0.0878	-0.06018	0.0186	0.0168	0.001783
6C	0.1243	0.0945	+0.0298	0.0247	0.0337	-0.0090
7C	0.1996	0.0872	+0.1124	0.1287	0.2902	-0.1615
8C	0.2391	0.2698	-0.0307	0.0255	0.1229	-0.0974
9C	0.2344	0.1608	+0.0736	0.1087	0.1374	-0.0287
10C	0.0931	0.0592	+0.0339	0.0341	0.0586	-0.02457

Experiment III

Pyruvates (um/ml)
(shock dogs)

	Before Shock			After Shock		
Dog	Art.	C.S.	A-V Diff.	Art.	C.S.	A-V Diff.
1S	0.0452	0.1714	+0.12613	0.0976	0.2642	-0.16660
2S	0.1258	0.1295	-0.0037	0.3596	0.3279	0.0317
3S	0.0784	0.1095	-0.0311	0.0704	0.1642	-0.0938
4S	0.0185	0.1146	-0.09608	0.0502	0.1807	-0.13044
5S	0.0390	0.1221	-0.0831	0.1456	0.2291	-0.0835
6S	0.1335	0.1801	-0.0466	0.0551	0.3690	-0.3139
7S	0.2539	0.1462	0.10762	0.1297	0.2363	-0.10656
8S	0.1822	0.2455	-0.0633	0.0579	0.1742	-0.1163
9S	0.1682	0.2385	-0.0703	0.0163	0.1194	-0.1031
10S	0.1861	0.1451	-0.04100	0.0148	0.3001	-0.28521

Experiment III

Lactates (mg/100ml)
(control dogs)

Dog	Before 1 Hour Art.	C.S.	A-V Diff.	After 1 Hour Art.	C.S.	A-V Diff.
1C	23.247	14.145	9.102	24.209	11.203	13.006
2C	18.423	19.239	-0.816	28.521	21.032	7.499
3C	43.133	31.849	11.284	46.448	28.597	17.851
4C	53.054	35.871	17.183	40.171	17.559	22.612
5C	52.044	44.430	8.214	30.998	25.582	5.416
6C	33.895	15.705	18.190	16.844	6.775	10.069
7C	42.703	32.408	10.295	41.479	32.446	9.033
8C	69.446	49.318	20.128	69.305	59.095	10.210
9C	69.933	52.221	14.712	61.118	49.113	12.005
10C	22.313	10.110	12.203	18.983	9.796	9.187

Experiment III

Lactates (mg/100ml)
(shock dogs)

	Before Shock			After Shock		
Dog	Art.	C.S.	A-V Diff.	Art.	C.S.	A-V Diff.
1S	41.170	37.447	3.55228	109.737	123.857	-14.12065
2S	91.139	101.827	-10.688	117.585	132.977	-15.392
3S	27.339	17.290	10.049	87.777	**	
4S	57.087	49.418	7.669	103.745	89.329	14.416
5S	44.294	26.803	17.491	81.214	91.164	-9.950
6S	11.499	10.097	1.452	109.024	100.536	8.488
7S	70.236	32.437	37.899	99.981	79.387	20.594
8S	30.361	30.724	-0.363	99.517	85.766	13.751
9S	61.828	50.321	11.5075	113.146	111.062	2.0843
10S	27.257	25.407	1.850	84.027	54.115	29.912

**sample lost

Experiment III

Dog	% O_2 Sat. Before Shock 1 Hour Art.	C.S.	After Shock 1 Hour Art.	C.S.	B.P. (mmHg) Before Art.	After Art.	Approx. Blood Loss (cc)
1C	95	43	96	47	80	80	
2C	97	43	97	43	115	112.5	
3C	99.5	36	100	40	105	80	
4C	99	37	99	36	80	85	
5C	99	34	99	36	105	90.5	
6C	98	33	96	34	120	127.5	
7C	97	35	99	36	71.5	70	
8C	93	53	92	51	75.5	66.5	
9C	94	53	95	57	90.5	90	
10C	93	42	92	42	113.5	125	
1S	99	45	98	29	120	35-39	700
2S	98.5	49	99	37	*	24-37	360
3S	99	36	99	25	100	28-37	620
4S	97	37	97	32	101	35-50	830
5S	100	43	99	21	131	21-30	600
6S	99	24	87.5	27	118	22-40	600
7S	99	36	98	33	89	36-47	600
8S	99	27	98	37	82	28-33	400
9S	94	49	94	44	77	19-30	250
10S	94	55	93	37.5	95	32-34	450

*partially occluded catheter, inaccurate reading

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